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OBSERVATIONS ON THE RELATION OF THE SYMPATHETIC NERVOUS SYSTEM TO SKELETAL MUSCLE TONUS[1]

FRANCIS DANA COMAN

From the Department of Anatomy, Johns Hopkins University

INTRODUCTION

The relation of the sympathetic nervous system to the activity of cross-striated muscle has engaged the attention of an increasing number of investigators during the past few years, especially following the recent advocacy of surgical intervention in certain of the spastic neuropathies. The rationale of the advised procedure is based upon evidence somewhat equivocal and yet upon which is built the concept of a dual innervation of skeletal muscle tonus by cerebrospinal and sympathetic components. In a survey of the rapidly growing literature on the subject one is impressed with the mass of contradictory data, the lack of agreement on the meaning of terms, particularly that of "tonus," and with the divergent conclusions drawn from practically identical observations. It is not within the scope of the present report to include a comprehensive critique of the work done in this field, yet a brief review of some of the conflicting evidence upon which the hypothesis of dual innervation is based might be of aid in evaluating the results of the present writer's investigation.

TONUS IN SKELETAL MUSCLE

A clear concept of the meaning implied in the term "tonus" in relation to cross-striated muscle is essential to an intelligible understanding of the question with which we are dealing. Unfortunately, the term itself is one concerning which there is almost notorious disagreement. Thanks to Sherrington (73), who has recently sketched the vicissitudes through which use of this term has passed since

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Galen's original reference to τονικαὶ κινήσεις, or tonic actions, which kept limbs in the position assumed, we have our most generally accepted conception of tonus as a postural reflex. As a result, however, of the variations in definition still held by certain authors, comparison of data in many cases is almost futile. Vulpian believed tonus to be simply a state of permanent muscular tension, a view which is more or less current (*e.g.*, Foix (25)). The word *tonus* seems to imply an associated tension, but this idea has been shown to be erroneous.

The greater part of our knowledge concerning skeletal muscle in the tonic state has been derived from observations on decerebrate preparations after the method first described by Sherrington (69). An animal submitted to transection of the midbrain in the region of the superior colliculi develops, after a short interval, a contraction of the antigravity musculature not only of limbs but of tail and neck. Under appropriate conditions the decerebrate rigidity thus evoked is maintained throughout a long period. If during this state the contraction of the extensor muscles is passively overcome, the rigidity suddenly gives way and without resistance the limb may be flexed or extended into any given position, which will be maintained without actual tension against moderate forces. It is this property of tensionless adaptation that led Sherrington (71) to express as "plastic tonus" the phenomena exhibited by skeletal muscle in decerebrate rigidity.

Langelaan (44), however, has used the term "plastic tonus" in a much more restricted sense. Attempting to analyze muscle tonus, he described the factors "contractile tonus," resulting from a state of feeble contraction, and "plastic tonus," a neuromuscular property spontaneously maintaining the position assumed through action of the contractile factor. Similar attempts at analysis have led to such terms as a "mechanical" or "Brondgeest tonus" as contrasted with a "chemical tonus" described by Barenne (3). For the sake of clearness in the following pages we will specify the meaning we wish to convey when using the expression "plastic tonus."

The conception of dual factors in muscle tonus has led to much speculation and to various contradictory assertions concerning the fundamental differences between tonic and tetanic contraction. It

is, of course, well established that less energy is required to maintain a state of tonus than one of tetanic contraction. Roaf (65), for instance, has shown that the CO_2 production in muscles in decerebrate rigidity is not perceptibly greater than that in curarized muscles, and as this finding was unaffected by sympathetic ramisection, it seems completely to refute de Boer's assertion (12, 13) that in the absence of sympathetic innervation decerebrate rigidity is tetanic in character. De Boer has further asserted that the prolonged contraction characteristic of the tonic state in general is similar to that seen in veratrinized muscle and is merely associated with a difficulty in removal of the products of metabolism. Such a concept of tonus, however, is at once invalidated by the demonstration of the same heat ratio for veratrine contraction as for ordinary tetanic contraction reported by Hartree and Hill (31).

Fundamentally, there must be a large degree of similarity between tonic and tetanic contraction. Pekelharing and Van Hoogenhuyze (59), in noting an increase in creatine in limb muscles after several hours of decerebrate rigidity, show that tonus involves an actual protein breakdown. Moreover, Adrian (1) has called attention to the similarity in electrical response in tonic and phasic contraction, following the work of Buytendyk (19) who found that muscles in a state of decerebrate rigidity and those in voluntary tetanus exhibit the same oscillatory galvanometric discharge. On the other hand, Kahn (37) reports the entire absence of action current in the forelimb of the clasping frog; and Frank (26) reports the production of plastic tonus of the Langelaan type in the completely denervated frog's limb through the action of nicotine. Such evidence as the foregoing must convince one of the need for more definite data, especially of a morphologic character. Certainly, an understanding of any of the phenomena of muscle tonus presupposes some knowledge of the structural units involved.

MORPHOLOGICAL ELEMENTS INVOLVED IN MUSCLE TONE

Duality of contractile fibres

The existence in certain invertebrates of distinct muscles for the performance of phasic and tonic contraction (von Uexküll's "motor"

and "catch" muscles), together with the widespread existence of white muscles capable of repeated, rapid contraction, and of red muscles capable of slow, powerful contraction, has led many investigators to assume the existence in general of two types of fibres in skeletal muscle. Again, as is not unusual, speculation has far outdistanced actual observations. Many theories have been offered, but only a limited number of careful investigations.

Ranvier (63), in 1887, described two distinct types of myofibrils in cross-striated muscle. The red fibres seen by him possessed more sarcoplasm than the white fibres, responded to excitation more slowly and with a longer latent period, and were associated with a certain peculiar arrangement of blood vessels, the significance of which he was unable to determine. Gruetzner (30), confirming these observations, considered one class of fibres to serve as an intrinsic support upon which the contractions of other fibres might be superimposed. Ten years later Bottazzi (16) developed the concept that the two contractile elements previously described consisted of myofibrils or sarcostyle concerned with clonic contraction, and sarcoplasm concerned with the maintenance of the contracted state. In other words, the tone of the sarcoplasm was believed to support the anisotropic sarcostyle in its quicker contraction.

Similarly, Roberts (66), in his studies on degenerated muscle, concluded that the brisk twitch of normal human muscle is the contraction of white fibres or sarcostyle, while the slow contraction seen in muscle following denervation is the contraction of red fibres or sarcoplasm which resists degeneration. He based this conclusion, strangely enough, on the observation of practically no difference in the rate of degeneration in the white and red muscles (gastrocnemius and soleus) of the rabbit.

More recently, Frank (26) has further affirmed the sarcoplasmic theory of Bottazzi concluding that in each unit of skeletal muscle is included an element of smooth muscle, the sarcoplasm, serving as substrate for tonic phenomena, while the striated fibril is concerned not only with quick contraction but with the summation of such contraction in tetanic contraction.

Duality of conducting fibres

Tschiriew (77), in 1879, and Bremer (18), in 1882, described both medullated and non-medullated nerve fibres terminating in motor end-plates in striated muscle. The endings of the non-medullated fibres, found for the most part in the aponeuroses of the muscle fasciculi, Tschiriew thought to be embryological forms of the medullated neuro-muscular apparatus. The central origin of the non-medullated fibres was, of course, not determined. Perroncito (60), in 1902, also described both medullated and non-medullated fibres terminating in skeletal muscle, believing the two types of nerve fibres to end in different types of muscle fibres. Whether the non-medullated fibres were ultra-terminal branches of medullated nerves, or whether they were branches of non-medullated vascular plexuses, was still undetermined. A few years later Boeke (6, 7), confirming the grape-like endings of the non-medullated fibres as Tschiriew described them, remarked their hypolemmal position. His diagrams show continuity of non-medullated fibres with those going to plexuses around neighboring small vessels. Although he insisted that these non-medullated fibres were not ultra-terminal branches of medullated nerves, neither he nor Botezat (15), who at the same time described medullated and non-medullated fibres ending in striated muscle in birds, was able to determine their central origin.

At length, by the use of degeneration methods on the extrinsic eye musculature following somatic denervation, Boeke (8, 9) claimed to have demonstrated that cross-striated muscles are innervated doubly through mutually independent somatic and sympathetic nerve endings having hypolemmal position and thus apparently conducting centrifugal impulses. In association with Barenne, further degeneration studies, in which the intercostal muscles in the cat were examined thirty days after intradural spinal root section, led Boeke (10) to the conclusion that the accessory fibres and end organs were indeed efferents of sympathetic origin. Agduhr (2) at the same time examined the limb musculature following section between the spinal ganglion and the white ramus communicans of roots of the brachial plexus. He reported that, while the majority of sympathetic fibres terminate in the hypolemmal position, many

of the fibres end in an epilemmal position. This observation proved only that sympathetic fibres are present in the musculature examined and not that individual muscle fibrils, as a rule, are so innervated. Boeke, however, considered the sympathetic fibres to be those concerned in the innervation of tonus, agreeing with the view previously expressed by Mosso (54) that the sympathetic innervation governed tone and slow contraction, while cerebrospinal innervation governed voluntary and quick contraction.

The work of Boeke, Agduhr, and Barenne has been criticized especially in regard to the histological preparation. Silver impregnation methods have been used exclusively: both Wilson (82) and Langley (47) have suggested that many of these so-called non-medullated fibres might possibly be finely myelinated fibres whose sheaths, resisting degeneration, had become impregnated with the silver. Moreover, it seems obvious that, since the sympathetic nervous system supplies the vessels of skeletal muscle, a plexus of sympathetic fibres will persist after somatic degeneration. It may be recalled in this connection that Ranvier (63) emphasized the highly vascular condition of the red fibres of skeletal muscle, and as Langley (47) points out, it is essential to determine whether fibres from this vascular plexus end under the sarcolemma of muscle fibres or whether they all end in connection with blood vessels. Both hypolemmal and epilemmal endings have been described. Hunter (32), while accepting the hypolemmal terminals of non-medullated fibres as sympathetic in origin, interprets the epilemmal endings described by Agduhr (2) as belonging to ultra-terminal twigs of somatic nerves, despite Agduhr's assertion to the contrary. Further, it must be determined whether somatic and sympathetic fibres terminate in the same or in separate myofibrils.

Upon finding a larger increase in fibrous tissue in musculature following sympathetic denervation than that noted after somatic nerve section, Hunter (32) concluded that this phenomenon represented selective degeneration and that it demonstrated the existence of two types of muscle fibres with specific innervation, the fibres presumably innervated by the sympathetic supporting the somatically innervated fibres concerned with voluntary and reflex contraction. This agreement with Mosso's doctrine seems unwarranted by the

evidence, for there is no proof that the sympathetic is directly concerned with muscle tonus, even should we admit that the increase in endomysium is a compensatory reaction. The metabolic changes following removal of the sympathetic innervation may more than account for the proportionate increase in fibrous tissue. Hunter, moreover, gives large weight to the recent demonstration by Kulchitsky (39) of medullated and non-medullated nerve endings in the skeletal muscle of the frog by methylene-blue and gold-chloride methods. This latter investigator reports that "each muscle fibre receives only one more or less complex motor terminal," a finding that awaits interpretation and confirmation.

RELATION OF SYMPATHETIC NERVOUS SYSTEM TO SKELETAL MUSCLE TONE

Hypothesis of exclusive control (Mosso and de Boer)

On the basis of the description of Perroncito (60) confirming the previous work of Tschiriew and of Bremer, mentioned above, Mosso in 1904 published his theory of dual innervation of cross-striated muscle by the sympathetic and cerebrospinal nervous systems. He expressed the belief that the non-medullated fibres described were sympathetic in origin and governed tonus and slow contraction, while the somatic fibres were concerned only with ordinary quick contraction. Some years later, following morphologic evidence (Boeke (6)) that the non-medullated fibres were actually of sympathetic origin, de Boer (11) performed unilateral section of the lumbar rami communicantes in the frog and observed a loss of tone in the affected hind limb. He concluded with Mosso that muscle tonus was governed exclusively by sympathetic innervation.

A number of observers, repeating de Boer's experiment on the frog, promptly reported negative results; Beritoff (5) concluding that tonic innervation, like every other reflex, is not only stimulated through cerebrospinal efferent neurones. but is completely and exclusively dependent on cerebrospinal coördinating centers. Kuno (40) likewise found neither loss of tone nor any difference in rate of stretching of the involved muscles under load, and he attributed de Boer's results to uncontrolled vasodilatation following sympathetic

extirpation. De Boer (14), however, repeated his experiment on the cat and found tonus in hindlimb and tail abolished. Again his work failed to be confirmed. Barenne (3) found a slight and transitory decrease in tone following sympathetic ramisection in both the frog and the cat. Negrin-y-Lopez and von Brücke (55) similarly reported the loss of tone transitory when present at all, and concluded with Barenne that de Boer's concept of tonus maintained exclusively by sympathetic innervation was untenable, most of the tonic impulses being conducted by cerebrospinal nerves. According to these findings, decerebrate rigidity is in no wise dependent on sympathetic impulses.

Meanwhile, in answer to the contention of Pekelharing and van Hoogenhuyze (59) that since tone involved the utilization of protein it must be somatic in origin, de Boer (14) claimed that sympathetic impulses caused the protein breakdown in sarcoplasm during the maintenance of the tonic state. But de Boer's supposition had little weight, since the process was unaffected by sympathetic denervation.

The concept of exclusive control gained a strong adherent, however, in Kuré and his co-workers (42), who found that following splanchnic nerve section the diaphragm was drawn up into the chest by negative pressure. So many factors are involved here that one hesitates to agree that this is evidence of direct control of tonus by the sympathetic system. Suffice to say, that the hypothesis of exclusive control of skeletal muscle tonus by sympathetic innervation not only lacks confirmation but has practically been universally abandoned.

Hypothesis of sympathetic control of plastic tone (Langelaan)

In 1915, Langelaan (44), in reviewing the evidence on the subject, came to the conclusion that there must be two distinct elements in muscle tonus; namely, "contractile tone," concerned with the assumption of posture and innervated by cerebrospinal efferents, and "plastic tone," concerned with the maintenance of the assumed posture and innervated by sympathetic efferent neurones. As mentioned previously, a somewhat similar concept was expressed by Barenne (4), who referred to "mechanical" or "Brondgeest" tonus as due to impulses received through somatic motor end-plates, and "chemical tonus" as dependent on sympathetic innervation.

The concept of a dual nature of the tonic state stimulated anew a concerted effort toward the solution of the innervation of tonus. The hypothesis of sympathetic control of Langelaan's "plastic tone," it will be seen, can be proved by any one of three general demonstrations, namely, (*a*) tonic state produced by stimulation of sympathetic nerves; (*b*) tonic state abolished by section of sympathetic nerves but intact cerebrospinal innervation; (*c*) tonic state maintained with intact sympathetic innervation but section of cerebrospinal nerves.

Subsequent experimentation has with slight modification attempted to fulfill one or more of the foregoing conditions.

a. Stimulation of sympathetic fibres. Attempts to produce a tonic state in skeletal muscle by stimulation of sympathetic nerves either directly or through the action of adrenalin has, with few exceptions, met with consistently negative results.

Direct faradic stimulation has been especially unpromising. Deicke (23), on stimulating the white and gray rami communicantes, found no effect on the form or the electrical response of the muscles involved. Uyeno (78), stimulating the stellate ganglion before and after decerebration, found no effect on the development or maintenance of the tonic state. Bottazzi (17), stimulating sympathetic fibres to the diaphragm following somatic denervation, found no tonic effect in that structure. On the other hand, Langworthy (49) has found that, following degeneration of the hypoglossal nerves, stimulation of the lingual nerve brought about a slow contraction of the tongue musculature (Vulpian's phenomenon). While he concluded this might possibly illustrate the stimulation of autonomic nerve fibres innervating skeletal muscle, the increased fibrillation of degenerating muscle and the great vasodilatation make one wish to rule out circulatory and catabolic changes. Further, Ginetsinsky (28) reports the restoration of fatigued muscle in the frog by direct stimulation of the sympathetic rami communicantes, likewise a case in which vasomotor factors must be considered.

The action of the sympatho-mimetic amine, adrenalin, in stimulating the myoneural junctions of postganglionic fibres has been studied with similarly contradictory results.

Oliver and Schäfer (57), in 1895, reported that the administration of adrenalin in the frog and the dog was followed by an increased

height of striated muscle contraction and its delayed relaxation. These observations have been cited recently as confirming a sympathetic influence on skeletal muscle activity despite their repudiation (Schäfer (68)) after several investigators, Panella (58), Kuno (40), and Takayashu (76), had found the drug to cause either no effect or an actual decrease in the height of muscular contraction. The restorative action of adrenalin on fatigued muscle, as reported by Panella (58), Cannon and Nice (20) and by Gruber (29), may be admitted, but although attempts have been made to control the vasomotor factors, the results fail to convince one that any element of a sympathetically governed tonus is involved.

Kuré and his co-workers (43) in 1922, continuing their observations on the tone of the diaphragm, report the local application of adrenalin to restore tone previously lost on section of the splanchnic trunks. On the other hand, Gayda (27) recently reports that adrenalin has no action whatever on striped muscle tone. His conclusion that skeletal muscle tonus is therefore not dependent on sympathetic innervation seems unwarranted, however, since not all of the phenomena known to be governed by the sympathetic nervous system are activated by adrenalin.

At best, the results of attempts to produce a state of tone in skeletal muscle by stimulation of its sympathetic innervation have been inconclusive.

b. Removal of sympathetic innervation. Apparently the most popular approach to a solution of the problem has been observation of results following sympathetic denervation with maintenance of an intact cerebrospinal innervation. Whether the sympathetic block is produced by actual extirpation or through the action of nicotine, the observed effects on the state of muscle tone have been contradictory in the extreme.

The original experiment of de Boer (11) resulting in loss of tone in the hindlimb following lumbar ramisection, as stated above, has never been confirmed. It has been repeated many times both according to the original procedure and with various modifications, but with equally negative results. Negrin-y-Lopez and von Brücke (55), Cobb (21), and van Rijnberk (64) agree that extirpation of the abdominal sympathetic trunk in the cat fails to modify in any way

the development or maintenance of decerebrate rigidity in the involved limbs. Kahn (37), observing the clasp reflex in the foreleg of the frog, found no alteration in tone following cervical sympathectomy. This was later confirmed by Spiegel (74). On the other hand, section of the cervical sympathetic trunk in the rabbit is reported by Ducceschi (24) to result in distinct loss of tone in the extrinsic ear muscles.

Saleck and Weitbrecht (67), in repeating de Boer's experiment, found the tonic effect slight and inconstant. They concluded that plastic tone (evidently of the Langelaan type, *i.e.*, "Tonus von Brückenstellung") was not decreased through sympathectomy, and that tonic stimulation was dependent not at all on sympathetic fibres but on cerebrospinal nerves. Langelaan (45), however, reported a partial but definite loss of tone in repeating the same experiment. But, at the same time, Takahashi (75) found neither tonic nor trophic effects on skeletal muscle following extirpation of the lumbo-sacral sympathetic trunk. This finding was confirmed and further elaborated by Langley (48) and Newton (56) who found unilateral abdominal sympathectomy to produce neither tonic nor heat effects. To balance this last evidence, however, we find that Foix (25) obtained partial but permanent tone loss following lumbar ramisection in the dog; and Hunter and Royle (32, 33, 34) report loss of "plastic tone" in decerebrate preparations of the goat and the pigeon following abdominal or cervical sympathectomy and report the relief of cases of spastic hemiplegia similarly by sympathetic ramisection. Kuntz and Kerper (41), in a preliminary report, seem to confirm the results of Foix and of Hunter on the dog and the pigeon. Kanavel and his co-workers (38), however, have been unable to find any tonic effect either before or after decerebration following sympathetic denervation in the cat. Kanavel, moreover, following a clinical study of cases in which according to Hunter and Royle sympathetic ramisection is advised, fails to find sufficient evidence to warrant this procedure, while even more significant is the previous report of Crothers (22) of rather disappointing results in cases operated upon by Royle himself.

The action of nicotine has been utilized in investigating the relation of sympathetic innervation to skeletal muscle tone. As is well

known, nicotine in small doses stimulates sympathetic ganglion cells, while larger doses may be sufficient to paralyze not only sympathetic elements but cerebrospinal neurones as well. It is important, then, that dosage be very carefully controlled and this factor always kept in mind when examining evidence.

Frank (26) in 1920, injecting nicotine subcutaneously into the frog, reported the production of a plastic tonus in the forelegs. In this condition the limb apparently exhibited a wax-like flexibility in preserving without great rigidity any position passively given it. This might appear to demonstrate the stimulation of sympathetic tonic activity, but as the same cataleptic tonus was produced in a limb in which the entire brachial plexus had been sectioned one finds difficulty in interpreting the results.

Uyeno (78), in 1922, found the application of nicotine to the stellate ganglion in the cat to be without effect on decerebrate rigidity. On the other hand, Kuré and his associates (43) report that nicotine applied to the coeliac ganglion causes abolition of tone in the diaphragm, and recently Ranson (62), in a preliminary note, reports a diminished rigidity *i.e.*, "loss of plastic tone," following the application of nicotine to exposed lumbar ganglia in the cat.

Even could we be certain just what action, stimulation or paralysis, a given amount of nicotine is going to exert, such evidence as that given above lacks weight because of such complicating factors as purity of the drug, comparative dosage, direct action on muscle, circulatory changes, and others.

c. Removal of somatic innervation. The results of observations on tonic changes in skeletal muscle, the cerebrospinal connections of which have been abolished but the sympathetic innervation of which remains intact, are more generally in agreement than those in either of the two preceding types of experiment. The one questionable factor here is the integrity of the sympathetic tracts.

Section of the anterior spinal roots, *i.e.*, somatic motor nerve section, has consistently resulted in complete loss of tone. Kuno (40) in 1915 found that division of the lumbar motor roots in the frog produced complete flaccidity of the muscles involved. Later, van Rijnberk (64) reported that section of the lumbar ventral roots in the cat prevented the development of decerebrate rigidity in the hind-

limb, a finding which Foix (25) has recently confirmed in the dog. In each of these cases, however, it is extremely doubtful that the sympathetic outflow has been left intact, and even if intact, whether such an element as the so-called sympathetically innervated plastic tone of Langelaan could be demonstrated in the involved limb.

Posterior root section, likewise interfering with the somatic reflex arc, has been shown repeatedly to prevent the appearance of decerebrate rigidity. It was first pointed out by Sherrington (69) in 1896 that deafferented muscles become flaccid in decerebrate preparations as a result of the abolition of tonic reflexes. Barenne (3), in 1916, and Saleck and Weitbrecht (67), in 1920, have reported "much greater loss of tone on posterior root section than on sympathetic ramisection." Frank (26) believes that tonus is dependent on the integrity of posterior roots, assuming that by way of these roots pass antidromic, centrifugal fibres of autonomic origin for the control of skeletal tone, and that preservation of a somatic reflex arc is not involved. This worker maintains that "tonus is controlled not from ventral horn cells but from the antagonistic interplay of autonomic nerve impulses."

Section of a nerve trunk itself includes, of course, section of both somatic and sympathetic fibres; hence any results of this procedure to be significant must follow some preliminary procedure such as selective section of somatic or sympathetic elements or the application of curara, nicotine, or similar drugs. Jansma (35), in 1915, found greater loss of tone on cutting the sciatic nerve than that following the previous section of the rami communicantes to this nerve. Conversely, Mansfeld and Lukacs (53) found greater loss of tone on cutting the sciatic nerve than that following previous paralysis of motor endings through the use of curara. The conclusions from these observations were that sympathetic fibres must be concerned in the maintenance of muscle tone.

Abolition of somatic innervation through the action of curara, presumably at the motor myoneural junction, has given little evidence of value. In many cases the experimental conditions have been so complicated, and the action of the drug has been dependent on so many factors, that the results are impossible to interpret.

Abolition of somatic afferent impulses by the use of cocaine in its

selective action on sensory elements has been found with all investigators to cause the disappearance of muscle tone. Weed (80), in 1914, caused a diminution in the rigidity of his decerebrate preparations by the application of cocaine to the superior vermis, whereby he was able to interrupt the reflex arc involved in this condition. Frank (26) bases his theory of antidromic autonomic innervation of tone on the finding that plastic tone of the nicotinized frog is abolished by the application of cocaine, and that tone in general is abolished by the action of cocaine or novocain. Kuré (43) has found that cocaine causes a relaxation of the tonus induced by adrenalin in a diaphragm previously freed from connection with the coeliac ganglion, an experiment difficult to interpret. Walshe (79) reports that novocain injected intramuscularly renders flaccid the spastic muscle of hemiplegia and relaxes the rigidity of paralysis agitans, but in no case impairs voluntary control or indirect faradic stimulation. Walshe thus concludes that tonus is dependent on the integrity of a proprioceptive reflex arc which Liljestrand and Magnus (50) had previously shown to be interrupted by novocain.

In summary, then, we find that stimulation of the sympathetic fibres to skeletal muscle has yielded only equivocal and unconfirmed results; a majority of observers find that elimination of the sympathetic fibres with preservation of cerebrospinal innervation of skeletal muscle has no effect on the tone of these muscles; and finally, there is general agreement that definitive loss of tone follows interference with the cerebrospinal reflex arc. Until there is universal agreement, however, further work must be carried on and the problem attacked from as many angles as possible. It is with this in mind that the present investigation was undertaken.

EXPERIMENTAL DATA

Rationale and material

The present series of experiments represent an attempt to observe the effect on the tonus of the forelimb of: (*a*) stimulation of sympathetic fibres passing to the limb; (*b*) ventral root section of spinal nerves of the thoracic sympathetic outflow; and (*c*) ventral root section of cerebrospinal nerves forming the brachial plexus. The re-

sults of these three investigations seemed to offer significant data in the establishment or refutation of the hypothesis of dual innervation of skeletal muscle tonus by sympathetic and cerebrospinal nerves. The somatic innervation of the foreleg in relation to the thoraco-lumbar sympathetic outflow furnishes a peculiary convenient anatomic basis for elimination of one type of innervation without disturbance of the other, a procedure much more difficult in the lower limb and which has rendered inconclusive results that have been obtained without control of this factor.

In the cat and dog the first ramus communicans albus leaves the cord with the first thoracic root, and the last of the thoraco-lumbar outflow leaves the cord with the third or fourth lumbar root. The secretory and vasomotor fibres for the forelimb leave from thoracic spinal roots iv to ix inclusive, rarely as far forward as the third spinal root, the maximal effect being produced by stimulation of the seventh. Stimulation or section of white rami higher than the fourth thoracic results only in secretory and smooth muscle changes in the head (particularly in the eye). Hence not only may the ventral roots of the entire brachial plexus, including the first or second thoracic, be sectioned without interfering with the integrity of the sympathetic innervation to the forelimbs, but section of the second or third to the ninth or tenth thoracic roots eliminates the sympathetic innervation of the forelimb without interfering with the integrity of the somatic innervation. Since the arrangement of both somatic and sympathetic nerve elements is subject to some variation, exploratory stimulation was employed in these experiments as a further precaution to insure complete removal or complete integrity of the given innervation.

The usual laboratory animals, the cat and the dog, were used both for convenience and because most of the data on skeletal muscle tonus have been obtained through work on these two animals. The material for the present series included 46 animals of which thirty-nine were cats and seven were dogs. All operative procedures were performed under ether anaesthesia and except in acute experiments under strict surgical asepsis. All acute preparations were controlled by careful autopsy to confirm the operative procedure and ascertain the condition of the spinal cord. All deferred preparations were

fixed by intra-arterial injection of 10 per cent formalin to preserve the tissue for further histological studies on the relation of sympathetic innervation to skeletal muscle through degeneration methods.

Observations of the effect on skeletal muscle tonus of stimulation of the sympathetic fibres in the thoraco-lumbar outflow

For this experiment animals having white ears, paws, etc., were selected to facilitate observation of vasomotor, pilomotor and secretory responses, and appropriate areas on the ears, paws, toes, etc., were shaved. Under ether anaesthesia a dorsal mid-line incision was made from occiput to mid-sacral region. Spinal musculature was divided and laminectomy performed from the third cervical to the second sacral vertebra. Bleeding was controlled; the exposed spinal cord kept moist and warm; and upon removal of epidural fat, the dura was split in the mid-line, allowing exposure and identification of appropriate nerve roots. By the use of cotton pledgets excess cerebrospinal fluid was removed from around the spinal root to be stimulated. A unipolar electrode was placed in contact with the ventral root just as its filaments unite to pass through the dura, and the root stimulated by increasing strength of interrupted faradic current. In some cases a fairly strong tetanizing current was necessary to cause a sympathetic response, but usually the sympathetic response appeared at the same time as the somatic.

In general, Langley's table (46) was confirmed for such sympathetic responses as salivation, movement in vibrissae, dilatation of pupils, retraction of nictitating membrane, blanching in ear and paw, sweating and pilomotor response in forepaw, etc. No sympathetic response whatever was elicited by stimulation of the third to the seventh cervical roots inclusive. In two animals there was slight dilatation of the pupil following stimulation of caudal filaments of the eighth cervical root. In no animal was there any vasomotor, pilomotor, or secretory response in the forelimb resulting from stimulation of roots higher than the fourth thoracic. Moreover, despite observation under the most favorable circumstances to rule out the vestibulo-cervical phenomena of Magnus and de Kleijn (52), in no case did stimulation of any of the thoraco-lumbar roots result in any detectable tonic effect in the limb musculature.

The results of this experiment, therefore, demonstrate the possibility of somatic denervation of the forelimb without interference with the sympathetic innervation; and further, that stimulation of sympathetic fibres, although causing known sympathetic responses, in no way affects the state of skeletal muscle tone.

Observations of the effect on skeletal muscle tonus of removal of sympathetic innervation

This experiment consisted in unilateral section of the first to the ninth thoracic ventral roots, the previous experiment having shown that the more caudal roots send no sympathetic fibres to the forelegs. After an interval varying from a few minutes, sufficient to allow observation of immediate tonic effects, to forty-eight hours, the animal was decerebrated. In no case, either before or after decerebration, was there any detectable difference in tonus, or in either voluntary or passive motion, in spite of section of somatic elements passing by way of the first thoracic root. One animal shown by exploratory stimulation to send somatic fibres to the foreleg from the second thoracic root was not included in this experiment.

The procedure was relatively simple. Following ether anaesthesia appropriate areas on the head and back were shaved and a mid-line incision was made from the vertebra prominens to the tenth thoracic spine. Skin and fascia were reflected, the axial musculature separated from the spinous processes, and laminae removed from the eighth cervical to the tenth thoracic vertebrae. Bleeding was controlled and the exposed cord kept warm and moist by appropriate measures. Following exploratory stimulation of exposed nerve roots on the left side, the first to the ninth thoracic ventral roots were cut. The animals at once showed ipsilateral constriction of the pupil, a slight reddening of the ear and relaxation of the nictitating membrane so that one-half of the pupil was covered. In those preparations in which decerebration was deferred, the operation was performed aseptically and, following closure, the animals were placed in a warm cage in uniformly excellent condition. Upon recovery from anaesthesia no tonic, motor, or reflex dissimilarity could be found in the forelegs either immediately or throughout the longest interval before decerebration. All limbs seemed to be used perfectly and no defect in muscular activity could be observed.

In decerebration the procedure followed was that originally described by Sherrington (69). For many reasons this method was preferred to that of basilar artery ligation as used by Pollack and Davis (61). The fundamental work of Sherrington (69, 70, 71, 72), of Weed (80, 81) and that of many others on decerebrate rigidity has been based on the transection method which has the added advantages of simplicity, accuracy, and opportunity for stimulation of given tracts exposed in the cut brain-stem. With ordinary precaution there is never any interruption in respiration, nor any significant haemorrhage, and consequently no symptoms of asphyxia or anaemia. Such decerebrate preparations may easily be kept many days with the aid of artificial heat, tube feeding, and passive evacuation of the urinary bladder.

In this series the animals were anaesthetized with ether; the carotid arteries were ligated and a trephine opening made under the temporal muscle on one side. The opening was enlarged with Horsley rongeurs, bleeding from the diploë being controlled with a preparation of bees-wax. Through a cruciate incision in the dura a small blunt spatula was inserted and carried forward along the bony tentorium in a quick, firm sweep. This procedure transected the cerebrospinal axis just posterior to the superior corpora quadrigemina. Meanwhile, the vertebral arteries were compressed at the base of the occiput, and after removal of the cerebral hemispheres through the trephine opening serious haemorrhage was rarely encountered. Following decerebration, within an interval dependent on the depth of ether narcosis, extensor rigidity began to develop, usually first in the elbows and knees, and subsequently in the hips, shoulders, and ankles.

Following the foregoing procedure, animals which had been deprived of the sympathetic innervation to one foreleg showed no bilateral differences in decerebrate rigidity, either in development or in degree of extensor contraction. Within intervals varying from two to twenty minutes beginning rigidity could be detected simultaneously in each elbow, thence appearing in knees, shoulders, ankles, etc., with no appreciable difference from one side to the other. Plastic tonus in the sense of ability of the limb to retain a position passively imposed was not impaired. Further, there was no detectable dif-

ference in the degree of homolateral augmentation or inhibition of rigidity resulting from unipolar stimulation of the cut brain stem in the region respectively of the red nucleus and of the area of inhibition described by Weed (80) in the mesial portion of the cerebral peduncles. Finally, all postural reflexes were equally active.

In this experiment there can be no doubt of complete removal of sympathetic influence on the foreleg; thus the observation of unchanged muscular activity following this removal demonstrates that sympathetic innervation can not be involved in the appearance of skeletal muscle tone.

Observations of the effect on skeletal muscle tonus of removal of somatic innervation

This experiment formed the major part of the present investigation, and inasmuch as interference in any way with sympathetically controlled factors was avoided, it has seemed more significant and more promising than either of the foregoing methods. The procedure employed consisted in intradural section of the ventral roots of the brachial plexus, the sympathetic innervation of the foreleg being left intact. The series included preparations decerebrated immediately following operation, preparations decerebrated twenty-four hours later, and those decerebrated two, four, six, eight, ten, twelve and fourteen days after the operation. In this way observation of immediate as well as remote and compensatory effects of somatic denervation was afforded. Under ordinary postoperative care there was complete recovery from shock, fear, gastro-intestinal and other disturbances, and those animals that were allowed to continue a week or more before decerebration uniformly showed perfect wound healing, were able to walk on three legs, held and moved the head normally in all directions, and otherwise exhibited perfect operative results. The post-operative interval was limited to fourteen days to exclude the complicating factor of muscle wasting. In this regard, however, it must be recalled that, according to the work of Roberts (66) and of Hunter (32), the hypothetically tone-governing red myofibrils of skeletal muscle survive degeneration even longer than the actively contractile white fibrils.

The operative procedure was somewhat similar to that of the preceding experiment, *i.e.*, following laminectomy and identification of intradural nerve roots, exploratory stimulation preceded complete ventral root section. Particular care was taken to avoid undue exposure or other trauma to the spinal cord or the posterior nerve roots. This precaution resulted in the absence in all deferred preparations of any visceral or sphincter disturbance, or any motor or sensory defect beyond the motor paralysis in the involved limb. In all cases, acute and deferred, the somatically denervated limb invariably showed complete absence of motor or tonic muscular activity.

Following ether anaesthesia and suitable preparation of the operative field, a mid-line incision was made from occiput to second thoracic spine. Skin and superficial fascia were reflected; cervico-scapular musculature, ligamentum nuchae, etc., were retracted intact; and the deep axial musculature was separated from spinous processes and laminae of the vertebral column. Following laminectomy of the third cervical to first thoracic vertebrae inclusive, the dura of the cord was exposed and split somewhat to the right of mid-line. Elevation of the dura on the left brought about a slight rotation of the cord, allowing excellent exposure and identification of the spinal nerve roots to be cut. Removal of superfluous cerebrospinal fluid by cotton pledgets permitted accurate unipolar stimulation of ventral roots and observation of the motor and sympathetic effects. Section of the ventral roots on the left close to their exit through the dura was performed by means of small blunt tipped scissors curved on the flat, as described for cervical sympathectomy by Jonnesco (36). Bleeding from vessels accompanying the roots was controlled by loose cotton packs in the interspaces, the ligamentum denticulatum having been severed to facilitate exposure. In this way rapid, easy and complete unilateral section of the ventral roots of the fourth, fifth, sixth, seventh, and eighth cervical, and first thoracic nerves was accomplished.

All bleeding and oozing being controlled, closure was started immediately. Deep, superficial, and subcutaneous fascia were reunited in layers by interrupted sutures of fine black silk, and final closure by through-and-through skin sutures provided perfect ap-

proximation without tension. Iodine and alcohol to the incision completed the operation, with good assistance the entire procedure generally taking about one hour. The animal was then placed in a warm cage and allowed to recover under careful postoperative attention. The procedure of decerebration subsequently performed has been described elsewhere.

The immediate effect of removal of somatic innervation, as mentioned above, was invariably a complete motor paralysis of the involved musculature. Before decerebration this paralysis was shown by a completely flaccid leg which apparently was quite useless, as the animal walked or hopped on the three intact legs. Pinching, faradic stimulation of footpads, or other noxious stimuli caused no change whatever in the flaccidity of the limb. The integrity of the sensory tract, however, was demonstrated in the protective reactions of other limbs or the withdrawal of the entire body in response to stimulation of the de-efferented limb. Likewise, integrity of the contractile elements was shown by lack of any dissimilarity in intact and operated limbs in the motor response to direct muscle stimulation. Beyond an occasionally constricted pupil on the operated side, no effect attributable to section of sympathetic elements in the first thoracic ventral root could be detected. There was no difference between the two forelegs in temperature, color, or other vasomotor manifestation, nor throughout the longest period preceding decerebration was there any observable difference due to muscular wasting.

Following decerebration the limb deprived of somatic efferents at no time showed the development of any postural reaction whatever, although normal decerebrate rigidity appeared invariably in the intact limbs. Noxious peripheral stimuli, and stimulation of the cut brain-stem were equally unproductive of any change in the denervated limb although, as before, the intact limbs reacted normally with augmentation of extensor rigidity. Similarly, passive adjustment of head and neck, while provocative of Magnus-de Kleijn phenomena in the intact foreleg, caused no reaction in the operated leg. With the animal in the dorsally extended position the three intact legs were maintained in rigid extensor contraction, while the de-efferented leg remained absolutely flaccid, merely seeking its own

center of gravity. With the animal suspended by rump and nape of neck, the de-efferented and flaccid leg hung at a higher level than the rigidly extended intact leg on the opposite side. Passive flexion or hyperextension of the operated leg resulted in none of the "shortening" or "lengthening" reactions of Sherrington (71). At no time by any means whatever could plastic tone in the sense used by Langelaan be detected in this de-efferented leg although observable in all of the other legs. Gentle pressure on the de-efferented footpad caused a contralateral reflex extension of the intact leg, but no ipsilateral response. Gentle traction applied to either de-efferented or intact foreleg produced bilateral progressive movements of the hindlegs. Faradic stimulation of the footpad of the operated leg resulted in no movement in the leg itself but extension or a scratch reflex in the opposite leg, together with movement of the vibrissae on the opposite side. The same stimulus applied to the intact foreleg caused quick withdrawal of the leg and crossed reflex movement of the vibrissae but no response whatever in the de-efferented leg. The crossed reflex movement of the vibrissae, in the absence of any facial muscle contraction would seem to demonstrate the integrity not only of somatic afferents but of sympathetic tracts as well (pilomotor to vibrissae).

In one preparation similar to one mentioned in the previous experiment, there was some doubt at the time of operation whether the second thoracic ventral root sent fibres to the forelegs. This was left intact, however, and the animal kept fourteen days before decerebration. The leg was never quite flaccid, although apparently quite useless. Upon decerebration, although markedly less than on the opposite side there was, in fact, unmistakable tonus in this limb, both "contractile" and "plastic" types of Langelaan being detectable. The operative area was reopened immediately and further dissection revealed a not uncommon anomalous arrangement of the spinal innervation in which the brachial plexus receives a fairly large branch from the second thoracic nerve root. Unfortunately, operative manipulation, loss of blood, etc., had inhibited rigidity in all the limbs and the animal was in such poor condition that it was thought best to sacrifice it immediately in order to secure good fixation of tissue for further histologic investigation. Hence, in this one case

of abnormal reaction, the effect of section of the second thoracic ventral root could not be determined. It is reasonable to assume, however, that as in all other cases of complete somatic denervation, tonus would have entirely disappeared.

DISCUSSION

As outlined above, the present investigation into the supposed relation of the sympathetic nervous system to skeletal muscle tonus has been conducted along three independent lines of attack and has resulted in findings showing remarkable accord.

In the first series, direct stimulation of appropriate sympathetic fibres led to no observable tonic effect. Faradic stimulation being employed here, it may be objected that incidental to the Nernst theory of nerve excitation, especially as developed by Keith Lucas (51), the proper rate and rhythm of electrical stimuli might not have been used. This objection, however, seems vitiated by the fact that the stimulus used was sufficient to induce practically all of the manifestations known to be sympathetic in character, such as pupillary, orbital, secretory, vasomotor, and pilomotor responses. A similar answer could be given to the objection that somatic impulses might mask sympathetic impulses in ventral root stimulation, for all of the above mentioned sympathetic responses were elicited, as in the simultaneous production of pupillary dilatation and tetanic motor responses in the foreleg on stimulation of the first thoracic root.

In the second series, removal of sympathetic fibres without the disturbance of somatic tracts resulted in no observable tonic effect. It must be admitted that the procedure employed here is open to objection in view of the necessarily included section of uninvolved somatic and visceral sympathetic fibres. As mentioned elsewhere, however, not all investigators agree that simple section of the white and gray rami includes complete section of all sympathetic innervation. In the presence of this factor, the normal development of tone in spite of undoubtedly complete removal of sympathetic influence seems all the more significant.

In the third series removal of somatic fibres without interference with the integrity of the sympathetic fibres led to such clear cut

abolition of tone in the involved musculature that this demonstration seemed especially significant and conclusive. Further, the simple method of ventral root section which has been described eliminated as nearly as possible many of the objectionable factors in previously reported investigations, such as lack of control of spinal cord or posterior root trauma, and lack of complete extirpation of one type of innervation either sympathetic or somatic without interference with the other type. Factors of muscular degeneration or of vaso-dilatation were likewise adequately ruled out. In one case of abnormal reaction it was subsequently demonstrated that an abnormal arrangement of somatic innervation was present, making it reasonable to assume that tone was only partially abolished because the somatic efferents were only partially removed.

Such results as the foregoing seem in perfect accord with Sherrington's concept of skeletal muscle tonus simply as a postural reflex under cerebrospinal control; none of the findings warrant the necessity of a distinction of elements in tonus, such as the contractile and plastic elements postulated by Langelaan; and certainly no evidence whatever was found to support the theory of a dual innervation of tonus by sympathetic and somatic nerve elements.

CONCLUSION

The results of the present experiment may be summarized as follows:

1. Stimulation of the sympathetic innervation to the foreleg failed to cause any tonic reaction.

2. Complete deprivation of sympathetic innervation to the foreleg failed to influence the normal development of tone either before or after decerebration.

3. Complete deprivation of somatic motor innervation to the foreleg was followed by complete abolition of tone in that leg both before and after decerebration.

Inasmuch as none of the conditions essential for proof of the sympathetic innervation of skeletal muscle could be observed, the conclusion is evident that there is no direct relation between the sympathetic nervous system and the development or maintenance of postural tonus in cross striated muscle.

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CHLOROMA

The Recent Literature and a Case Report

DORSEY BRANNAN

From the William H. Singer Memorial Research Laboratory and the Allegheny General Hospital, Pittsburgh, Pennsylvania

The recent pathological study of a case of chloroma or chloroleukemia prompted me to examine certain papers, including those of Lehndorff and Burgess. Lehndorff has tabulated all the cases of chloroma reported up to 1910 and divided them into lymphoid and myeloid varieties. Burgess, on the other hand, maintains without exception the myelogenous origin of this comparatively rare disease. Because of these two opposing points of view regarding the underlying nature of the disease, it seemed to me worth while to undertake a review of all the reported cases since Lehndorff's paper, with especial reference to the pathogenesis. A few other cases have been included in order to make the story more complete. A tabulation of cases has been omitted and only the salient features will be briefly discussed.

LITERATURE

The gross anatomical features of this disease were first detailed by Burns in 1823. His case presented classical characteristics; exophthalmos from green retro-orbital and lacrymal gland tumors, green tumors of the paranasal sinuses and dura, together with an unusual greenish discoloration of the cornea. Balfour, in 1835, reported the second case in a man 18 years of age with olive-green cranial, dural, orbital and sinus masses. The next case was by Durand-Fardel in 1836. King in 1853 described the fourth case and gave the malady its name, chloroma. Aran in 1854 studied the fifth case which showed extensive cranial and dural masses. Durand-Fardel and Aran both commented upon the occurrence of the green masses in the dura and the latter first pointed out, as well, the association of the tumors with the cranial bones.

Contrary to the general statements in the literature, Waldstein in 1883, and not Huber, made the first blood studies. Waldstein recognized the leukemia and anemia in his case, but did not correlate the two, and even considered the bone marrow findings apart from the blood changes. Von Recklinghausen made no cytological blood studies, but merely mentioned the similarity of the histological pictures of chloroma and leukemia. Dock, in 1893, on the basis of blood and other studies, first pointed out the relationship of chloroma and leukemia, and Dock and Warthin, in 1904, definitely established this point.

Notwithstanding the varied and often confusing terminology, especially used by the Germans, chloroma prior to 1903 had been generally accepted as essentially a primary disease of the lymphoid tissue. In this year Türk described the first myeloid case, in a man with myelogenous leukemia, with green growths along the spine and in the kidney. The bone marrow was green. Sternberg, in 1904, reported the second, and Klein and Steinhaus in the same year reported the third myeloid case. The nature of the disease in each instance was unquestionable, in the presence of myelogenous leukemia and the myeloid structure of the green tissue.

Thereupon, in 1905 Sternberg divided chloroma into two groups, namely, lymphoid and myeloid; based on blood and histological as well as on gross details. In 1909 Pappenheim classified the disease into hyperplastic chloroma and chlorosarcoma, with related intermediate forms. He regarded leukemia, chloroma and myeloma as somewhat similar disease processes of the haematopoietic system.

Lehndorff, in 1910, tabulated all the cases up to that time, a total of 73, of which 56 were lymphoid and 17 myeloid chloromas. He, however, classified among the lymphoid chloromas all the incompletely studied cases, as well as those so designated by the authors, but at the same time questioned the occurrence of so many lymphatic cases. Poor hematological methods and the generally accepted lymphoid nature of chloroma were undoubtedly primarily responsible for the slow recognition of myelogenous cases.

Following the observations of Türk, Sternberg, Klein and Steinhaus and others, the lymphoid cases began to diminish, and in 1912 Burgess brought forth strong and convincing reasons to show that chloroma

was always a myeloid process. He pointed out that many so-called lymphoid leukemias, if more completely studied, would prove to be acute myelogenous cases. The essential point in differentiation of the leukemias, as he made clear, resolves itself into the identification of the large mononuclear cells of the blood and those composing the tumors in chloroma. Askanazy has logically argued the occurrence of only myeloid chloromas, and a few others have expressed a similar belief.

Many of the present-day authors of textbooks, such as MacCallum, Naegeli, Ewing, Aschoff, Wood in Delafield and Prudden, and others, divide this disease into the two groups, the lymphoid and the myeloid. Martelli adds an endothelial variety and cites Fabian's case as an example, although Fabian himself apparently did not so consider it. In 1916 Sternberg grouped chloroma with leukosarcoma as well as with leukemia. Mallory alone of the textbook authors considers it a part of myelogenous leukemia, while Ordway and Gorham apparently lean toward this view.

The writer has reviewed all the cases reported since Lehndorff's paper, of which there were 74 including the case reported below. Of these, 49 were definitely myeloid and so diagnosed by the authors, but eleven of these were more or less atypical and border-line cases. Only one case was stated definitely to be lymphoid. The remaining 25 cases were very poorly studied and doubtful; indeed, some were probably not chloroma at all. Two of these were reported as giving a negative oxidase reaction.

At the outset the cases of Allison, Coronia, Catalnia, Gould and LeWald, Monsi, Tresilian, Nasso and Pearson's second case, because of insufficient data, may be dismissed as very doubtful, if chloroma at all. In none of these cases was there an autopsy or a biopsy, and green-colored tissue was therefore not demonstrated. The clinical features of certain of these cases could well have been those of chloroma, but because of the absence of green tumor tissue, they are not to be considered chloroma, and at best remain questionable. The chloromatous nature of Martelli's and of Ravenna's case, both of which came to necropsy, was also open to grave doubt, and apparently neither was chloroma. Excluding these 14 doubtful and the 4 border-line cases, there remain 56 recent cases of chloroma together with the 73 cases of Lehndorff's tabulation, which make a total of 129 cases of chloroma, up to 1925, about which there can be little or no question.

In the majority of the 49 cases considered to be of myeloid origin the diagnosis was based primarily on the blood picture, the histological structures of the tumor tissue and bone marrow. Except for a few cases of aleukemia, there was in all, definite acute or chronic myelogenous leukemia. In a few instances the authors did not emphasize or mention the classification, but the differential leucocytic counts were sufficient to place them in the myelogenous class. In the vast majority of cases, the cells composing the green tumor masses and bone marrow, as well as those in the blood, were large undifferentiated non-granular mononuclear cells, the myeloblasts; with or without a few or many neutrophilic and eosinophilic myelocytes. The eosinophilic myelocytes were especially numerous. The oxidase or peroxidase test was done on the white blood cells, or on the tumor and bone marrow tissue, or on both, in 17 cases, including the case below; in 15 of these it was positive. The cases of Askanazy and Hirschfeld were the exceptions, but on morphological findings they were regarded as myelogenous.

Hochhaus, in 1911, reported a case considered by him as one of lymphatic chloroma, with but one differential blood count; the large mononuclear cells he regarded as lymphocytes. He omitted the oxidase test. The cases of Lewis, Bruce, Kramer and Birmberg, Wynter and Lakin, and Bedell's second and third cases were apparently regarded as lymphoid by the authors on the basis of the reported differential blood picture and the interpretation of the sections, notwithstanding the presence in some of myelocytes; but the classifications were not definitely stated. The microscopic details of certain of these cases were very meager. In none of the above was the oxidase test tried on the white blood cells, tumor tissue or bone marrow. The cases of Sattler, Roberts and Bierring are open to similar criticism.

Ashby and Sellers reported a case confused with mastoiditis, with only one leucocyte count of 5100, 67 per cent of which they considered lymphocytes. The oxidase test on the tumor tissue alone was negative, but they did not state the technique used. Schlagenhaufer reported a characteristic case of chloroma, emphasizing the green color of the uterus. In this instance the only oxidase test was made on the tumor tissue and was reported as negative. The Schultz-

Winkler technique was employed. In neither case was the oxidase test applied to the white blood cells, and in the latter case there was no differential blood examination. The bone marrow was neglected in both.

One gets the impression from the literature that aleukemic forms of the disease were common. This assumption has developed, no doubt, on the basis of incomplete blood studies, since several cases are reported with only one, two or three blood counts, which were often within normal limits. The cases of Bramwell, Eichhorst and perhaps Hirschfeld probably were aleukemic forms. On the other hand, those reported by Darnall, and by Hoppi, Bedell's fourth case, and the case of Harada and Nagai show that, if sufficient white blood counts has been made, a definite and often well marked leucocytosis might have been found. In Darnall's case the leucocytic count ranged from 98,000 to 7420; in Hoppi's case, from 4800 to 50,000; in Bedell's case from 6200 to 519,000 and in the case of Harada and Nagai from 8800 to 48,500. Other cases reveal a similar variation in the white blood counts. Those of Flohr and Suganuma can hardly be considered aleukemic with but two white blood counts each, 18–20,000 in the former and 16–18,000 in the latter. The leucocytic count in the various reported cases ranges between wide limits. Burgess gives 1,880,000 as the highest, and 3200, in the case of Pissavy and Richet, was the lowest observed in the recent literature. The leucocytosis of chloroleukemia is relatively lower than that of the typical leukemias in general.

The characteristic color associated for the most part with the tumors varied from pale to dark green, often the former in the more acute cases. A noteworthy feature of the color was the prompt fading in the presence of light and air, but in tissue fixed in formalin it could generally be temporarily restored by the addition of hydrogen peroxide. Recently, Meyer and Berger state that the color may be preserved by placing the green tissue in glycerin. The nature of the green color is very obscure and no one has offered an explanation which has withstood criticism. Some authors have thought that the presence of eosinophilic cells was responsible, but this is very doubtful, as the relationship of the eosinophilic cells and the intensity of the color were not constant. This was especially noticeable in my case.

Practically all authors, except perhaps Meyer and Berger, were agreed that the chemistry of the color was unknown. They found some evidence of absorption bands at 645 and 490. One finds here and there in the literature the suggestion that it perhaps was a lipochrome. Askanazy considers the green color peculiar to myeloid cells and points out the occurrence of greenish-colored pus in support of his idea.

In this connection it is well to mention the work of Jacobaeus and Boots. In 1909, the former found that the sedimented white blood cells from a case of chloroma became green and faded in the light. The massed white blood cells from six other cases of myelogenous leukemia and several normal bloods gave no such color. Boots, in 1917, apparently unaware of the experiments by Jacobaeus, described a green color in the sedimented white blood cells from a case of myelogenous leukemia. Ordway and Gorham have also observed the formation of a green color in white blood cells of myelogenous leukemia. A greenish tint of the blood serum was also regarded by Jacobaeus and a few others as important.

The specific etiology of the disease is just as obscure as the chemistry of the color, and nowhere in the literature has anyone ventured an explanation. The leukemic and neoplastic properties were accepted by all the recent authorities except Sternberg. He regarded acute leukemia, with or without green tumors or green bone marrow, as an infectious disease. Chloroma is far more common in males than in females and is especially frequent in the young. A few cases have occurred in persons in the fifth and sixth decades.

Clinically, the symptoms described were essentially those of leukemia; namely, insidious onset, progressive weakness, undernutrition, pallor and hemorrhages; frequently with enlarged liver, spleen, and lymph nodes, and often with pains in the long bones and the sternum. The anemia was almost always considered as secondary; it was often severe and occasionally regarded as aplastic. The more characteristic clinical features were due to the tumors, which showed a peculiar predilection for the cranium, orbits, paranasal sinuses, ribs and sternum. Progressive exophthalmos followed by blindness, symptoms of intracranial tumors and cord lesions, nasal, throat, and oral symptoms were especially common, as were also flat extracranial masses. Very

often the disease ran an acute course. It invariably ended in death. Irradiation was used in a few cases and probably gave some benefit, but it was never curative. Fever was present in many cases. The urine analyses were unimportant, but green-colored urine was found in one case (see Lehndorff's tabulation). Bence-Jones bodies were not a feature.

In Huber's and Simon's cases the early manifestations were those of malignant breast tumors, and a mammectomy was done in each. Manini's and Bierring's cases had circumscribed green skin nodules, and green infiltration of the skin was recorded in two cases by Hitchman, and Firth and Ledingham. The cornea in Burns' case and the sclera in that of Ashby and Sellers were green. The prominent feature in Bruce's case was a lesion of the gums, an intra-pelvic sacral tumor in Darnall's case, and a neoplasm of the neck in that of Paulicek and Wutscher.

The post-mortem examinations usually revealed wide-spread green growths, generally firm or hard, which showed a marked predilection for the periosteum, the dura and the ligamentous structures especially of the trunk. Erosions of the cranial bones in relation with the dural tumors was a common secondary effect. Enormous flat infiltrating masses were found in many instances along the spine and filling the sacral concavity. Periosteal involvement of the bones of the extremities, though described, was perhaps not so common, while in only a few instances were these bones exposed. Rib and sternal masses were frequently encountered. The bone marrow, generally red or grayish red and always hyperplastic, excepting in the few instances mentioned below, had green patches in the cases of Paulicek and Wutscher, Mieremet, Sakaguchi and others. Apparently all the marrow was green in the cases of Türk, Emden and Rothchild, and Eichhorst. Several authors have described direct continuity of the bone marrow and the periosteal tumors, especially in the spine. The tumors in general, however, it appeared, arose from the localized growth of the leukemic deposits or metastases. From a survey of the reported cases practically no organ or tissue was exempt, but tumors were infrequent in the pancreas, thyroid gland, thymus, lungs, intestines, stomach and urinary bladder, and they were practically absent in the brain or spinal medulla. Splenomegaly and enlarged liver, as

found in leukemia proper, were often noted, but green tumors or patches in the spleen were very infrequent. In Durand-Fardel's case there was a green mass in the spleen. Green tumors or green patches in the liver and especially in the kidneys were fairly common. The chloromatous tissue in the organs was occasionally circumscribed, and seldom projecting above the capsules. Greenish discoloration of the supporting tissue especially of the organs has been noted several times and was present in the case reported below. Diffuse involvement of the tonsils and pharynx has often been observed. The lymph nodes of a regional group or groups were frequently enlarged and a generalized enlargement was not uncommon. The green color of the lymph nodes in a given case was not always uniformly distributed. Occlusion of large vessels by green masses has been noted by Aran, Askanazy, and others and probably occurred in Foot and Jones's case. Pleural effusions were common and constituted important pathological findings. The effusions probably resulted from pleural involvement, especially in relation with the periosteal infiltrations of the ribs.

The two cases reported by the Japanese authors, Harada and Nagai, and Sakaguchi, include extensive metabolic studies. An occasional case was complicated by tuberculosis and syphilis.

The cells of the tumors, infiltrations, bone marrow and the white blood cells were large, undifferentiated mononuclear cells designated as myeloblasts or large lymphocytes. On the other hand, many of the cases had in addition a few or many myelocytes especially the eosinophilic ones. Roman describes in his third case large numbers of basophilic cells. Mitosis has been frequently described and pictured, and definite invasions have often been observed. In the majority of cases, practically all the organs showed the characteristic infiltration found in leukemia. In general, these same structural details were true for the atypical cases. The same cells forming the tumors, and lying in the vessels, have been frequently commented upon.

The overgrowing and crowding out of erythrocytic cells in the marrow by the immature white blood elements has been emphasized by Doch and Warthin, as the underlying cause of the anemia. This feature was well marked in my case. Hemoglobin-containing cells were never found in the tumors.

The presence of hemosiderin in the liver, spleen, lymph nodes and bone marrow has been noted several times and was also found in my case. It no doubt indicates blood destruction.

A few atypical cases have been recorded which are worthy of comment. Pissavy and Richet, in the case of a woman of 40 years, suffering from weakness and anemia, found a relatively low white blood count ranging from 3200 to 18,800 per cubic millimeter. The predominating white blood cells were for the most part unidentified mononuclears, with a few myelocytes. Green ovarian and sternal tumor masses were found at autopsy, but the bone marrow was not hyperplastic.

In Firth and Ledingham's case of a boy of 12 there were leukemia, apparently myelogenous, a thickened and greenish-colored scalp, and colorless infiltration of the dura and periosteum by mononuclear cells. No tumors were found. The bone marrow in this instance was probably not hyperplastic.

In Rosenbaum's case there was myelogenous leukemia with green bone marrow, greenish-colored medulla of the kidney, and a greenish tint to the liver and spleen. The liver contained a small whitish nodule, and a large white retroperitoneal mass was also a feature.

The case of Roccaville was also atypical in so far as the distribution of the green color was concerned. The orbital and extracranial masses were green, while the dural thickening, exposed at operation, was gray. The large intradural tumor was green externally, but apparently because of central necrosis the interior had lost its green color. (The loss of the green color in the presence of necrosis is not to be regarded as unusual.)

Butterfield's first case presented green-colored kidneys, but tumors were absent. The blood contained immature myeloid cells. In his second case the bone marrow was not hyperplastic and the enlarged lymph nodes were the only structures that had a green color. There were no tumors.

Eichhorst's case presented apparently an aleukemic blood picture, with white blood counts from 3500 to 5750; the bone marrow was green and there were no tumors. Such cases as Butterfield's, Firth and Ledingham's, and Eichhorst's might be regarded as border-line ones, because of the absence of tumors.

Several cases were reported without green color of the tumors, but otherwise characteristic. The question arises as to the classification of these cases. It is difficult to draw a line between myelogenous leukemia with colorless tumors and chloroma proper, however, in cases of myelogenous leukemia with characteristically located tumors, without green color, especially in the orbits, dura, about the cranial bones, periosteum and ligamentous structures in general, in which the tumor tissue is composed of myeloid cells; one is forced to regard such cases as closely related to chloroma or border-line cases at least. The pathological process in these cases is undoubtedly essentially the same as in classical chloroma, but the unqualified term chloroma should be reserved for those with green color.

Rowe and Hirschboeck described a case of myelogenous leukemia in a child of 18 months, with white myeloid tumors over the cranial bones, face and ribs. There were also white nodules in certain of the organs.

Pagniez and associates reported a case of myelogenous leukemia in a boy of 10 years, treated by irradiation. The wide-spread flat tumors in relation with the bones were white. There was in addition exophthalmos and nasal obstruction from growths.

Hirschfeld's patient was a boy of 8, with large, white, facial and orbital masses of myeloid tissue. This patient received irradiation therapy and Hirschfeld thought that this treatment had probably destroyed the green color.

Recently Moore has described a most remarkable case in a man, with white myeloid tumors of the humerus, femur, ribs, along the aorta and in the spinal dura. Early in the disease an upper extremity was amputated and shortly thereafter large tumors developed in the incision. The spleen was greatly enlarged and also presented tumor masses. The blood and bone marrow were typical of myelogenous leukemia. A large percentage of basophilic myelocytes was found in the blood on two occasions. This patient had been treated by irradiation.

To this intermediate group perhaps also belong the cases of Leber, Birk and others.

CASE REPORT

History. Walter E., a white boy, aged 14, was admitted to the Allegheny General Hospital, on the neurological service of Dr. W. H. Mayer, December 31, 1923, and died May 26, 1924.

The family and past histories were unimportant, aside from the fact that the patient had been a diphtheria carrier four years previously.

His chief complaint was paralysis of the left side of the face. The illness began in September, 1923, with pain in the ears. Later frontal headaches appeared, often accompanied by vertigo, sufficient to prevent sleep. In December he first noticed a swelling on the left temple followed by a left facial paralysis, and limitation of movements of the mandible. Diplopia soon developed and vision of the left eye became greatly impaired. His hearing had been poor for some time and by December his ability to hear was almost lost. Pain over the mastoids developed but there was no history of otitis media.

Physical examination. The patient was described as a poorly nourished, and pale, white lad.

Extending from the lateral edge of the left orbit and over the temple was a flattened, hard, and slightly painful tumor mass about 4 x 2 cm. and elevated about 1 cm. Slight exophthalmos was noted. The pupillary reactions were normal. A left facial paralysis was described. Herpetic lesions were present at the angle of the mouth on the right side. Marked impairment of hearing was found by air conduction but bone conduction was apparently satisfactory.

The posterior cervical lymph nodes of the left side were palpably enlarged, as were also the epitrochlear nodes. The axillary, inguinal and femoral groups of lymph nodes were not enlarged. The tonsils were enlarged.

The chest was thin with marked supraclavicular and infraclavicular fossae. The lungs were negative. The heart was not enlarged, but a blowing systolic murmur was heard over the precordium. The pulse rate and rhythm were normal and the blood pressure was 110/58.

The abdomen was described as negative.

The genitalia and the extremities were negative.

The abdominal reflexes were present and equal, but the knee-jerks were not obtained. Plantar stimulation gave a normal response. The biceps reflexes were not obtained. There was no incoördination of movements.

Course. The patient's condition remained essentially unchanged until January, about four months after the onset of symptoms, when there developed bleeding from the gums. On January 23rd, symptoms referable to the ears and mastoids suggested an otitis media. He gradually became weaker, cachectic and more anemic. The systolic blood pressure fell from 110 to 90 and below, and the diastolic pressure could no longer be obtained. The exophthalmos gradually increased and became more marked on the left side. Ophthalmoscopic examination showed considerable elevation of the nerve heads with atrophy. The flat mass on the left side of the head gradually became larger and other flat hard masses

appeared on both sides, and over the forehead. The head became unusually large and markedly asymmetrical, resembling somewhat that of a hydrocephalic. Headaches became so severe that the patient often cried out with pain. Mentally, however, he was alert. The spleen became just palpable a month before death. The lymph nodes of the posterior cervical chains, as well as a few axillary and inguinal nodes, became moderately enlarged and firm. On May 10th the knee-jerks were recorded as absent, but the biceps and the abdominal reflexes were obtained. The latter were not increased and former were sluggish. Plantar stimulation gave a normal response, and the Oppenheim leg phenomenon was negative. The patient died quietly on May 26th, five months and twenty-six days after admission or approximately ten months after the onset of symptoms.

The pulse rate was generally rapid throughout, ranging from 100–110 per minute for weeks at a time. At times it was as high as 120–130 per minute, usually accompanied by some fever. The temperature was irregular and generally rose at some time during the day to 100°–101°F. At times it was as high as 103°–104°F. and seldom within normal limits after the first few days.

Cytological blood examination 1–4–24. R.B.C. 3,450,000; W.B.C. 12,000; Hb. (Sahli) 50 per cent; C.I. .7.

1–12–24. W.B.C. 36,800; Diff. (200 cells counted) : P.M.N. and Myelocytes 96.5 per cent, Lymph. 5 per cent, and L. Mon. 3 per cent. Polymorphonuclear leucocytes and myelocytes were not differentiated, as the former were atypical and essentially metamyelocytes.

1–14–24. W.B.C. 32,500.

1–17–24. R.B.C. 3,432,000; W.B.C. 32,000; Hb. (Sahli) 50 per cent; C.I. 7. Diff. (200 cells counted) : P.M.N. 9 per cent, Lymph. 6 per cent, L. Mon. 85 per cent, Red blood cells showed moderate anisocytosis and pallor.

2–5–24. R.B.C. 2,250,000; W.B.C. 37,000; Diff. (200 cells counted): P.M.N 3.5 per cent, Lymph. 20 per cent, L. Mon. 67 per cent, Myelocytes 8.5 per cent, Unclassified 1 per cent; 6 normoblasts seen. Anisocytosis and pallor of red blood cells. (Lymphocytic percentage probably too high.)

3–3–24. R.B.C. 2,650,000; W.B.C. 35,950; Diff. (200 cells counted): P.M.N. 1 per cent, Lymph. 2.5 per cent, L. Mon. 2 per cent, Myeloblasts 94.5 per cent. Marked anisocytosis and slight poikilocytosis; 9 nucleated red blood cells found.

3–4–24. W.B.C. 34,000.

4–5–24. W.B.C. 35,400; Diff. (200 cells counted): P.M.N. 2 per cent, Lymph. 9 per cent, Myeloblasts 86 per cent, Unclassified 3 per cent. Peroxidase test showed 88 per cent of myeloid cells; 6 normoblasts seen, anisocytosis and poikilocytosis of red blood cells, platelets sparse and very small.

5–6–24. W.B.C. 39,900; Diff. (200 cells counted): P.M.N. 4 per cent, Lymph. 6 per cent, Myeloblasts 87.5 per cent, Unclassified 2.5 per cent. Peroxidase test revealed 91.5 per cent myeloid cells. Anisocytosis and poikilocytosis of red blood cells. No nucleated red blood cells recorded.

Urinalysis. The urine on admission was negative as were numerous other examinations, aside from a trace of albumin and a few hyaline casts. Later in the course of the disease a few white blood cells were found. One atypical reduction test was reported, but sugar was never demonstrated.

Blood culture, 1–16–24. No growth.

Blood Wassermann negative.

Spinal fluid examinations, 2–4–24. The fluid obtained under increased pressure was clear and sparkling and contained two cells per cubic millimeter. Globulin, colloidal gold and Wassermann tests negative.

Blood chemistry, 2–13–24. N.P.N. 26.5 mgm. per 100 cc. blood. Blood sugar 104 mgm. per 100 cc. blood.

Roentgenological examination, 12–31–23. Slight evidence of increased intracranial pressure was found. The skull was of normal thickness, except for some thinning over the temples.

1–16–24. Heart and aorta were found normal. Some infiltration was found in the hilus of each lung, but it was not considered tuberculous.

1–30–24. Cloudiness was reported in the left mastoid.

2–5–24. No abnormalities were discovered in the long bones.

3–24–24. The fronto-parietal sutures were found slightly separated, and the skull markedly thinned. The soft tissues of the face and over the skull were thickened; the paranasal sinuses and orbits showed an unusual density. There was marked evidence of increased intracranial pressure, but no definite tumor could be demonstrated.

5–19–24. The skull was reported essentially the same as on previous examination.

Autopsy

The autopsy was performed by the writer two hours after death.

External appearance. The body was that of a cachectic, pale, white boy. The head was unusually large and asymmetrical due to diffuse, low, flat, irregular and hard tumor masses most marked on the left side. On the forehead above each eye were two rather prominent but poorly defined and moderately elevated masses, the one on the right side measuring 3 x 4 cm. On the left side the frontal mass was continuous with a large, irregular, hard and deep-seated, temporal mass, which extended downward upon the cheek to the lobe of the ear, even more prominently than the temporal mass and was slightly movable. Under the scalp were numerous, irregular, slightly elevated, poorly defined and hard masses particularly well marked laterally. There was a most marked exophthalmos greater on the left side, and both pupils were widely dilated. The skin over the forehead and temporal tumor masses was tense but not adherent. The nose, ears and mouth, aside from a few petechial hemorrhages in the latter, were negative. The bones of the face were otherwise negative.

The lymph nodes of the posterior triangles of the neck, right axilla and in the groins were slightly enlarged, firm and movable.

The neck, trunk and extremities, aside from marked wasting of the tissues, were negative.

The upper pole of the left testicle was slightly enlarged, firm and irregular.

Abdominal cavity. The liver extended 2 cm. below the costal margin and was lying free, as was also the gall-bladder. The spleen was adherent over its lower pole. The urinary bladder was greatly dilated and clear urine was easily expressed. The stomach was contracted and bound down posteriorly by adhesions, which almost obliterated the lesser peritoneal cavity. The peritoneum was everywhere smooth and glistening without hemorrhages. There was no excess of peritoneal fluid.

Thoracic cavity. In the anterior layer of the parietal pericardium were small, circumscribed, firm and dirty green tumors, none larger than 6 mm. in diameter. The heart was slightly dilated and lying free in the thin-walled pericardium. The supporting tissue between the pulmonic artery and aorta was greenish.

Both pleural cavities contained bloody fluid; 500 cc. in the left and 300 cc. in the right cavity. The right apex was found densely adherent.

The thymus was a small, flabby, spongy and greenish-discolored organ lying in edematous mediastinal tissue. There was practically no mediastinal fat.

Skeletal structures. The ribs and the spine were covered with a hard, thick and light green tissue. The ribs were marked out by broad green bands due to periosteal infiltration. Circumscribed small, dirty green and flat masses were scattered about on the under side of the sternum. Over the spine the green tissue was 5–20 mm. thick. Over the left side of the 7th or 8th thoracic vertebral body was a projecting firm green tumor 15 x 20 x 10 mm. The lumbar muscles and crura of the diaphragm were extensively infiltrated by the firm green growth. Large slices of an almost cartilage-like green tissue were stripped from the vertebrae. The sacral concavity was filled with a similar hard green growth, but the other pelvic bones were negative.

Heart. Weight 200 grams. The organ was normal in configuration, but flabby and edematous. Numerous petechial epicardial hemorrhages were scattered over the ventricles. The right chambers were dilated and the foramen ovale patent. The left chambers and valves were negative. Small, irregular, and characteristically mottled patches of fat were scattered through the flabby myocardium.

Right lung. Weight 500 grams. Apical adhesions and scarring were found but tubercles were absent. Numerous small and large fresh hemorrhages were scattered about over the pleural surfaces and in the parenchyma, with a moderate degree of edema. Scattered hemorrhagic patches of consolidation were widely distributed over the cut surfaces. The hilic lymph nodes were negative.

Left lung. Weight 250 grams. Aside from apical adhesions it was essentially the same as the right lung.

Trachea. This structure had a greenish discoloration externally, but no tumors.

Thyroid gland. This organ was moderately enlarged. The right lobe was much larger than the left, due to small adenomata.

Liver. Weight 1100 grams. The organ was normal in shape and configuration,

smooth and light chestnut brown in color, with uniform cut surfaces. The supporting tissue was pale green in color, but not increased in amount. There were no tumors. There was but little blood in the liver and the lobular architecture was not differentiated. The gall-bladder and ducts were negative.

Spleen. Weight 120 grams. The organ was a trifle enlarged, firm and smooth. The pulp was reddish-brown in color. Lymph follicles were not seen. There were no hemorrhages, green tumors or discoloration.

Pancreas and suprarenal glands. These organs were negative.

Right kidney. Weight 110 grams. The organ was smooth externally with here and there small, non-elevated, poorly circumscribed, round green patches; the largest measuring 6 mm. in diameter. On section, the green patches were found entirely limited to the cortex and roughly triangular in shape with the base at the renal capsule. The parenchymatous tissue was otherwise pale and dull. About the calices and vessels the tissues were greenish in color. There were also a few cortical hemorrhages. The pelvis and ureter were negative.

Left kidney. Weight 100 grams. This organ was similar to the right, but the green cortical patches were smaller and less numerous.

Urinary bladder. Aside from dilatation the bladder was negative.

Prostate gland and seminal vesicles. These structures were negative.

Testicles. Occupying the upper third of the left testis was a smooth, hard, sharply circumscribed, pale green tumor, 15 mm. in diameter. The tunica albuginea was slightly roughened but not especially bulging. Otherwise the organ was negative. The right testis and epididymis were negative.

Stomach and intestines. The gastrointestinal tract was not abnormal.

Cranium and brain. On stripping back the scalp the skull was found covered by a diffuse, low, irregular, hard and olive-green growth 10–12 mm. in thickness. The growth over the temples was 15–20 mm. thick, but the mass of the left cheek was not disturbed. Only a few thin and pale brownish temporal muscle fibers remained. The skull was found considerably thinned and eroded over the outer table; the diploë was reddish in color. The inner side of the cranium was free from the growth, but the sutures were slightly separated and the bone overlying the dural tumors was eroded.

In the dura, along the superior longitudinal sinus, were numerous irregular, flat, greenish masses, 2–4 mm. in diameter, with an irregular, narrow central zone of greenish discoloration overlying the sinus. The dura over the left occipital lobe had a large flat slightly elevated green tumor, 5 x 3.5 cm. Over the posterior and under surfaces of the left temporal lobe was a large, irregular green tumor, 4.5 x 3 x 1.5 cm. which, together with a second growth, 3.5 x 3 x 1.5 cm., adjacent to the cerebellum, filled the cerebellopontine space. A large finger-sized ridge of green tumor tissue extended along the left outer edge of the tentorium. On the right side were two large dural masses, approximately the size of the two large tumors of the left side. One of these was over the tip of the occipital lobe and the other lateral to the cerebellum. A few smaller masses were also scattered about. The tumor masses were densely adherent to the base of the skull and had deeply

eroded the bones. The growths were mostly on the inner side of the dura, however, and were buried deeply in the soft brain tissue. The tumor surfaces in relation to the brain tissue were somewhat lobulated, while externally the surfaces were roughened, especially those about the base. On section, the tumors were of fine texture and an olive-green color throughout, and without degenerative changes. They were non-encapsulated and hard. The dural sinuses had not been invaded by the tumor. There was no infiltrating growth in the dura of the base of the skull. Aside from marked erosion in the posterior fossa, the base was negative.

Brain. Weight 1430 grams. The cerebral convolutions were uniformly and markedly flattened, and large cavities marked the site of the dural tumors. The pia and arachnoid were smooth and glistening, but had been destroyed underneath the tumors. The cranial nerves and vessels were not grossly altered. The brain on section was otherwise negative. The cerebrospinal fluid was clear.

The spinal cord was not removed.

Orbits and paranasal sinuses. When broken into, the orbital fossae were found filled by a firm olive-green growth in which were embedded the optic nerves and other structures; the adipose tissue had been completely replaced. A similar growth filled the ethmoid, sphenoid and frontal sinuses, as well as the mastoid cells. The middle ears had not been invaded.

Lymph nodes. The mesenteric, mediastinal, axillary and inguinal groups were greatly enlarged, firm, homogenous and yellowish green in color. The largest were the mesenteric nodes which measured 18–20 mm. in length. The tracheobronchial nodes were only slightly greenish and a trifle enlarged. The retroperitoneal lymph nodes were buried in the hard green tissue described.

Bone marrow. The marrow from the femur was abundant, friable and deep red in color. The periosteum was negative. The marrow of the ribs was red but not especially abundant.

Muscles. Besides showing the greenish infiltration of the crura of the diaphragm and of the lumbar muscles, the musculature was otherwise pale and wasted.

Blood. The blood was very pale and thin, and clotted slowly.

Color. After a few hours the green color of the tumors and infiltrations faded. It could be restored once, several days later, in the tissue which had been fixed in formalin, by the addition of hydrogen peroxide.

Blood culture. A culture of the heart's blood revealed hemolytic streptococci.

Microscopic examination

Fixation—Zenker's solution; stains—methylene blue and eosin.

Tumor tissue. The dural tumors were composed of a uniform growth of closely packed, oval or rounded and large mononuclear cells. Only at the edge of the tumors was dural tissue found. The stroma of the tumors was sparse and the blood vessels were small and inconspicuous. No necrotic patches or hemorrhages were found, but a few phagocytes were seen containing hemosiderin. The tumor cells

presented a well-defined limiting membrane. The cytoplasm was pale, uniformly stained, fairly abundant and for the most part non-granular. An occasional cell with fine or coarse granules was found. The cells were a little larger than plasma cells. The nuclei, more or less vesicular, were prominent, oval or round, with one or more nucleoli. A few contained much chromatin and an occasional mitotic figure was found. Practically all the cells were well preserved and resembled myeloblasts. Perhaps a few cells could be regarded as neutrophilic myelocytes, and scattered neutrophilic polymorphonuclear leucocytes in various degrees of differentiation were found. A few eosinophilic leucocytes and large myelocytes were easily recognized.

Tumor tissue from along the spine, from the concavity of the sacrum and from the extracranial masses, presented a similar well preserved neoplasm of closely packed, large, polyhedral and nongranular mononuclear cells. The sections also presented patches and strands of widely separated and atrophic muscle fibers and markedly infiltrated tendinous and ligamentous structures. In these sections eosinophilic cells were sparse. A few lymph nodes were found, which were not unlike those described below.

Cerebral cortex. In the sections of the eroded areas a few shrunken pyknotic large pyramidal cells and a few scattered nononuclear phagocytes were found lying in a delicate reticulum. The leptomeninges were missing. No invasion of the nervous tissue by the tumor was observed.

Skull. The outer table was irregularly eroded and the periosteum had been practically destroyed by infiltrating mononuclear cells. The diploë contained only a moderate number of similar nononuclear elements but seemed otherwise negative.

The mastoid air cells were filled with tumor cells which were lying between the bone and the epithelium. Chronic inflammatory changes were uncertain and at most very slight.

Testicles. The orchitic tumor was made up of closely packed large mononuclear cells with numerous patches of eosinophilic leucocytes and myelocytes. The eosinophiles were mostly limited to the periphery of the growth. Here and there, but only about the edges, atrophic seminiferous tubules were found. There were a few granular tumor cells and occasional neutrophilic polymorphonuclear leucocytes were seen. A few regular and irregular mitoses were conspicuous. Scattered degenerating cells were fairly numerous, but necrotic or hemorrhagic foci were absent. Only the inner layers of the tunica albuginea were infiltrated and elsewhere the edges of the tumor were poorly defined, as practically all the testicular tissue contained strands and masses of tumor cells. The atrophy of the seminiferous tubules was due to pressure, as practically none had been invaded.

Kidneys. The sections of the cortical tumor areas were not unlike those just described in the testis. The kidney capsule formed a limiting membrane externally, while, as in the testicular tumor, the edges in the parenchymatous tissue were not well defined. Kidney tissue was absent in the centers of the largest patches and atrophic about the periphery. Eosinophilic cells were numerous. The kidney

tissue otherwise showed a moderate degree of parenchymatous degeneration. Beautiful collections of large mononuclear cells, identical with those in the tissues, were found in the blood vessels. The kidney pelves were moderately infiltrated by similar cells.

Spleen. This organ was diffusely infiltrated and packed with the same large mononuclear cells and a liberal sprinkling of eosinophilic leucocytes and myelocytes. The lymph follicles were small and the small lymphocytes were in marked contrast to the large, paler, mononuclear cells. Endothelial leucocytes were packed with hemosiderin granules. The spleen contained very little blood and the vessels were filled with large tumor cells. No necrosis, hemorrhage or blood islets were found.

Lymph nodes. The sections of all the lymph nodes mentioned above showed the same picture, varying only in degree, namely, a marked infiltration of the same large mononuclear cells which had practically replaced the lymphoid tissue. A striking feature was the large number of eosinophilic myelocytes and leucocytes, which were more numerous than in any other tissue examined. The sinuses were packed with the large pale mononuclear cells. Large phagocytes laden with hemosiderin granules were numerous. The tracheo-bronchial lymph nodes had a few fresh hemorrhages, but not much black pigment. As in the spleen, the small deeply stained lymphocytes were in marked contrast with the large tumor cells. The lymph follicles were small and atrophic.

Bone marrow. The sections of bone marrow of the femur presented a diffuse growth of the same beautifully preserved, uniform, mononuclear elements, apparently very early myeloblasts with many small nests of eosinophilic myeloid cells. Neutrophilic polymorphonuclear leucocytes were practically absent and neutrophilic myelocytes were sparse. Small islets of erythrocytic tissue, showing numerous nucleated forms and pale red blood cells, were only fairly numerous, having been almost crowded out. Phagocytes containing hemosiderin were plentiful. Occasional isolated megakaryocytes were seen. Mitoses of the myeloblasts as well as scattered degenerative changes were numerous.

Liver. The striking feature in the sections of the liver was the extensive central necrosis which in many instances involved a third of the lobule. Numerous neutrophilic polymorphonuclear leucocytes were intermingled with the necrotic cells. The same mononuclear cells with many eosinophiles were found scattered about in the capillaries. There were no blood islets. A few of the stellate endothelial cells contained hemosiderin granules. The remaining liver cells were very pale and swollen and many contained fat vacuoles. The nuclei of the liver cells were generally large and pale.

Lungs. A terminal bronchopneumonia was characterized by a leucocytic and mononuclear, fibrinous and thick fluid exudate filling the bronchioles and neighboring alveoli. The hemorrhage was fresh and somewhat apart from the pneumonic patches. Here again the supporting tissues of the bronchial walls were infiltrated with numerous large mononuclear cells, as described above. Even certain of the alveoli contained these cells as did also the blood vessels.

Heart muscle. Fibers here and there contained numerous tiny fat vacuoles and the supporting tissue was edematous. A few isolated, small foci of the mononuclear tumor cells were to be seen in the epicardial tissues and between the muscle fibers. About some of these there were fresh hemorrhages and fibrin deposits.

Thyroid. The adenomata were made up principally of large, dilated, irregular alveoli, lined with a low epithelium. The alveoli, both of the adenomata and throughout the gland, were unusually large and the epithelial cells were low cuboidal. Colloid was abundant. Tumor cells were found infiltrating the capsular tissues only.

Thymus, prostate gland, seminal vesicles and adrenals. These tissues were all infiltrated by the same mononuclear cells with a sprinkling of eosinophilic myelocytes and leucocytes, but were otherwise negative.

Pancreas. The pancreas alone was free from infiltrating cells and showed no abnormalities.

Peroxidase test. Goodpasture's technique; used also on blood smears. Frozen sections from the formalin preserved spleen, dural and cranial tumors were made and in each instance the reaction was strongly positive. The cytoplasm of many cells contained blue granules, but other cells took a more or less diffuse greenish-blue color. Practically all the cells gave a positive reaction.

EXPERIMENTS

In view of the simple experiments of Jacobaeus, Boots, and Ordway and Gorham on the formation of green color by the myeloid cells, the writer performed 4 similar experiments from 4 cases of chronic myelogenous leukemia.

The blood was collected in sodium oxalate containers, mixed and placed in the refrigerator. The specimen was allowed to stand from five to seven days. In only one instance did a green color appear, and this was slight and very pale.

Experiment by Dr. Taylor: The green tissue was passed through a meat chopper and dried by suspending in acetone. Extraction with ether gave rise to a greenish-yellow solution which, on evaporation, left a reddish brown fatty residue. This residue, dissolved in various fat solvents, showed absorption from the green to the violet end of the spectrum. The boundary was not sharp. The more concentrated solutions were reddish-brown in color and on dilution became greenish yellow. The color of the pigment did not change appreciably in several months.

COMMENT

This case in most respects was typical of chloroleukemia. The patient was a young male, suffering from myelogenous leukemia with gradually developing and characteristic, hard, flat cranial tumors, exophthalmos with marked visual and fundus changes, facial paralysis, impairment of hearing and symptoms confused with mastoiditis

and otitis media. There was marked roentgenological evidence of intracranial pressure, orbital and sinus growths.

The white blood counts, which ranged from 12–39,900, were relatively low for leukemia but rather typical of chloroleukemia. If there had been no other than the first leucocytic count, this case might have been considered as aleukemic. It is also of interest to note the changes presented by the leucocytes, which at first were largely metamyelocytes but later mostly myeloblasts. A few of the latter were very small and otherwise atypical. The cells listed as large mononuclears in the fourth blood examination were certainly myeloblasts, as shown later by more careful study. The marked secondary anemia, with evidence of regeneration of the erythrocytes, was a characteristic feature. Unfortunately, there were only two available hemoglobin determinations.

The autopsy findings of extensive green tumors over the skull, in the dura, paranasal sinuses, orbits, along the spine, sacrum and ribs in relation especially with the periosteum and ligamentous structures were distinctive enough. Erosion of the bones and pressure atrophy of the tissues, by tumors and infiltrating cells, were well marked in this case. The bone marrow was red and very abundant especially in the femur, but the examination was not extensive enough to exclude possible green patches. The slightly enlarged and firm spleen, the pale, yellowish-green and enlarged lymph nodes, renal and testicular masses and greenish discoloration of the supporting tissues constituted other but no less important pathological findings. The autopsy easily explains the symptomatology, except perhaps the impairment of hearing. This no doubt was due to involvement of the auditory nerves.

Microscopically, the tumors, bone marrow, organs and the blood cells in the vessels showed the same large non-granular mononuclear cells, the myeloblasts with few or many eosinophilic and a few neutrophilic myelocytes and locally differentiating polymorphonuclear leucocytes. Basophilic myelocytes and leucocytes were absent. The infiltration of the organs was essentially the same as that in myelogenous leukemia. The large number of eosinophilic myeloid cells in the lymph nodes and in the edges of the orchitic tumor were noteworthy findings and show the fallacy of associating the green color

with these cells. The accumulation of the tumor cells within the lung alveoli was a peculiar feature, but it has been observed before. It should also be noted that the mitoses were not especially atypical, and tumor giant cells were not found. Megalokaryocytes were found only in the marrow. The myelogenous character of this case has been clearly proven.

The central necrosis of the liver was apparently associated with the streptococcus infection.

SUMMARY

It is clear from a survey of the literature that the better studied cases of chloroma for the past 20 years have been of myelogenous origin. Only the older cases, and recently those not completely examined, have been considered lymphoid. All of the latter cases lacked either a complete blood study, or a histological examination of the green tissue and bone marrow, or a combination of these. The oxidase test was omitted in all but two. One cannot help being impressed by the fact that many authors have considered all large, non-granular, mononuclear cells as lymphocytes, even in the presence, in a few instances, of a fair number of myelocytes. Such data leave one in grave doubt as to the correctness of the diagnosis in these cases.

In this connection it may be emphasized that the ultimate classification of leukemias in general depends primarily upon the identification of the pathological leucocytes, especially the non-granular, mononuclear elements. In all cases except in chronic myelogenous leukemia the cells consist of non-granular mononuclears in various degrees of differentiation. No case has been found in which the predominating cells were considered small lymphocytes similar to those of chronic lymphoid leukemia. Would it not be logical to expect such cases if acute lymphoid cases occur?

Since one cannot rely altogether on the morphology of these pathological white blood cells, the oxidase or peroxidase test should always be tried before reaching a decision. Comparatively few authors have used the oxidase test in chloroma, but, where employed, the reaction has been positive in all but four instances. In two of these, the cells were proven to be of myeloid origin on other evidence. The technique of the oxidase test was stated by but one writer,

Schlagenhaufer, who employed the Schultz-Winkler method. In view of the pitfalls of the oxidase reaction, two otherwise poorly studied cases with negative oxidase tests cannot be used as reliable evidence in favor of the lymphoid origin. Of late years it has become well known that very early myeloblasts may, unfortunately, not give a positive oxidase reaction. The peroxidase stain of Goodpasture gives promise of much better results and should be applied to the tumor tissue as well as to the blood in all doubtful cases. The proteolytic ferment test probably could also be used for differentiation of the cells in uncertain cases. This test has been of value in the study of the leukemias and should be utilized in chloroleukemia. To conclude, as many have done, that one is dealing with a lymphatic chloroma when the essential tests and examinations have been omitted, or only partially performed, is unwarranted.

The involvement or absence of involvement of the lymph nodes means nothing for or against the lymphoid origin, as a study of the reported cases reveals. The enlargement of the lymph nodes, when not complicated by other processes, was due to the large mononuclear cells such as were found elsewhere. The infiltrated myeloid cells in chloroma have the power to proliferate in lymph nodes as well as in other tissues. Myeloid metaplasia in lymph nodes, as described by Roman, stands apparently unconfirmed, and is open to a different interpretation.

It seems very probable that the green color, characteristic of chloroma, is bound up with the myeloid cells, and every one is familiar with green-colored pus made up largely of neutrophilic polymorphonuclear leucocytes. Recently, the writer found two large intra-abdominal abscesses filled with bright green pus which promptly faded upon exposure to the air and light. Myeloid cells from leukemic blood have in a few instances formed a green color but this is not constant.

As the matter stands today, there is no indisputable evidence in favor of the lymphoid origin of chloroma. Since chloroma is a leukemic disease, it may be properly considered as a sub-variety or form of myelogenous leukemia and in this sense it is an entity. The neoplastic properties of true chloroma cannot be questioned. This is strong evidence in favor of the neoplastic nature of leukemia in general.

Because of the incomplete studies in the case reports, the evidence is not altogether favorable to the common occurrence of an aleukemic form of the disease, but apparently a few such cases have occurred. On the other hand, an aleukemic stage in a given case has been fairly common. Cases in which but one or two leucocytic counts were made, these falling within low limits, cannot be considered as aleukemic forms of the disease. Where the data were available, there apparently was a relationship between the low leucocytic counts and absence of hyperplastic bone marrow.

The disease may manifest itself in various degrees in addition to a leukemic blood picture. Some cases show only green bone marrow, others only green color of the organs or tissues. Such cases are to be regarded as atypical. The characteristic feature, as pointed out above, is the green tumor. In certain of the classical cases the marrow may be partially or entirely green. Still others have occurred in which all of the tumors were not green or not of the same intensity of color. Next apparently stand the cases without green color of the tumors, but which are otherwise characteristic. There is, then, no sharp line of separation between myelogenous leukemia and chloroleukemia.

There is little justification for the use of long and compound terms, especially employed by the German as well as by a few American writers. Chloroma or chloroleukemia seems sufficient.

CONCLUSIONS

The following conclusions seem justified.

1. Chloroma or chloroleukemia is a myelogenous process; an unusual form of myelogenous leukemia.

2. Aleukemic stages of chloroma were common, whereas proven, true aleukemic forms of the disease were very rare.

3. Transitional or border-line and atypical cases occur, which emphasize the close relation between myelogenous leukemia and chloroma.

4. A classical case of chloroma has been described.

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PLATE 1

FIG. 1. Natural size color reproduction of one of the dural tumors.

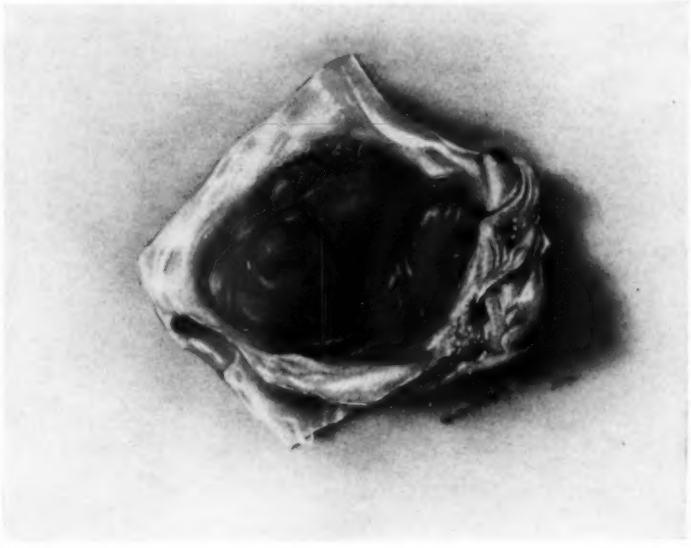


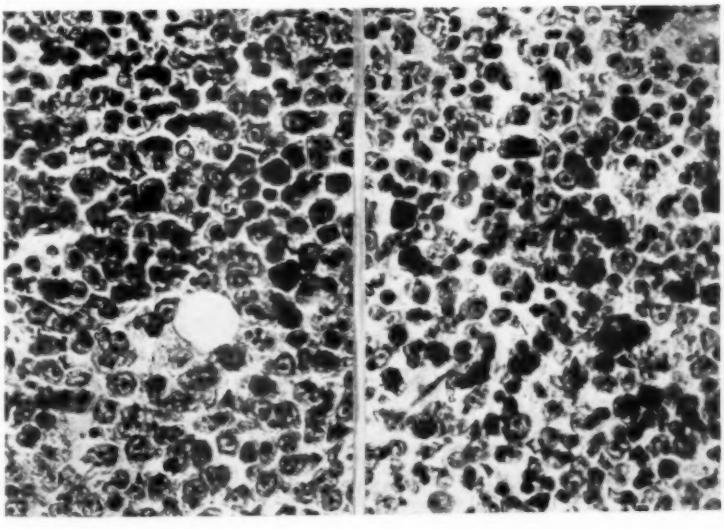
FIG. 1

PLATE 2

FIG. 2. Photograph of the patient 15 weeks before death. The bitemporal and left facial masses, swellings of the eyelids and dilated vessels of forehead are well shown. Condition much more exaggerated at death.

FIG. 3. Bone marrow from the femur, made up mostly of myeloblasts. In *a* there is a small islet of nucleated red cells at the top, and in *b* numerous eosinophiles.

FIG. 2



(b) (a)

FIG. 3

PLATE 3

FIG. 4. Section of one of the myeloid dural masses. Observe the differentiating polymorphonuclear neutrophilic leucocytes at the right edge.

FIG. 5. Section of one of the temporal masses, with parallel rows of myeloblasts infiltrating the temporal muscle. The muscle fibres have been destroyed in this area. A large eosinophil in center.

FIG. 6. From one of the mesenteric lymph nodes, showing the large number of eosinophilic cells; the lymphocytes have been replaced by the myeloid cells. The lymph sinuses are also partially filled with myeloid cells.

FIG. 7. Section of the spleen, showing the marked myeloid infiltration. The myeloid cells are in contrast with the lymphocytes of the small follicle.

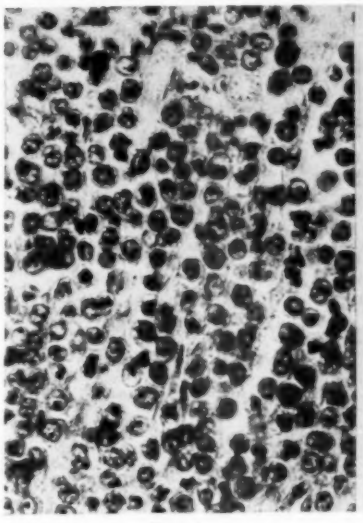


FIG. 4

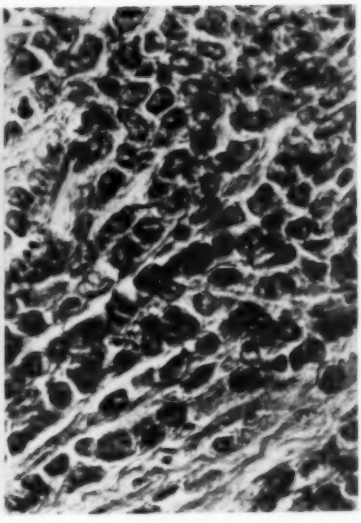


FIG. 5

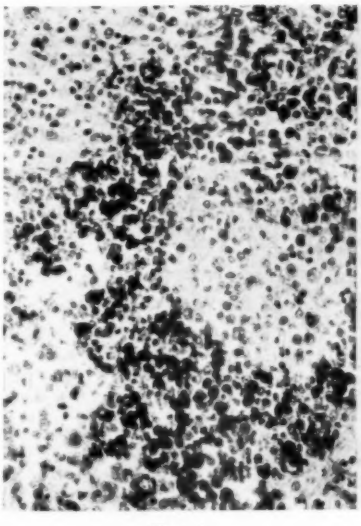


FIG. 6

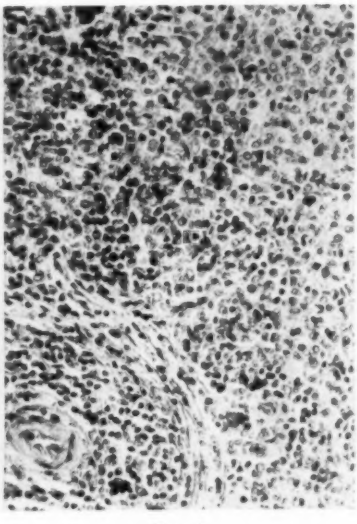


FIG. 7

INTERAGGLUTINATION OF MATERNAL AND FETAL BLOOD IN THE LATE TOXEMIAS OF PREGNANCY[1]

WILMAR M. ALLEN, M.D.

From the Obstetrical Department of the Johns Hopkins Hospital and University

In the literature dealing with the problem of the etiology of the late toxemias of pregnancy, particularly of eclampsia, we find numerous theories, not one of which, however, has been so definitely substantiated as to be generally acceptable. And yet several are sufficiently plausible to stimulate further work. Of these, that of interagglutination of maternal and fetal blood is perhaps the most immediately attractive as susceptible to experimental proof or disproof; and more especially so since previous work has made it seem probable.

Of those who have considered blood incompatibility as of etiological significance in the late toxemias of pregnancy, Dienst (1), Murray (2), McQuarrie (3), and Gruhzit (4) have reported serological studies of maternal and fetal bloods.

Dienst, in 1905, was the first to propose definitely that eclampsia is essentially the result of incompatible fetal blood entering the maternal vascular system. He performed cross-agglutination tests with the bloods of 118 mothers and their newly born infants. The technique is not described. In seven patients with eclampsia and two with albuminuria, incompatibility was found in every one (100%), whereas in normals it was present in approximately 14 per cent. Evidence of communication between fetal and maternal circulations was also adduced by the recovery of methylene blue from the maternal urine, after injecting the cord vessels with a solution of this dye immediately following delivery of the child. In all of the cases of toxemia such a communication existed but it was lacking in all of the incompatible normals.

Shortly afterwards, Liepmann (5) severely criticized Dienst's conclusions and methods, and in 1908 Dienst (6) admitted the in-

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conclusiveness of his own data and devoted himself to the fibrin ferment theory.

Murray also investigated the hematoxic nature of the toxemias of pregnancy, but found no interagglutination in two cases of albuminuria and in two of eclampsia.

In this clinic, McQuarrie studied the iso-agglutination phenomena in 140 normal, 16 definitely toxemic, and 24 mildly or potentially toxemic women and their infants. Of the whole series of 180, 44 (24.4 per cent) were incompatible. Of the 40 patients definitely or potentially toxemic, 30 (75 per cent) had blood incompatible with that of their infants. Incompatibility was present in only 10 per cent of the 140 normals. Careful microscopic technique was used. Of the infants 46.6 per cent had no evidence whatever of iso-agglutinins or iso-agglutinogens. McQuarrie was conservative in drawing conclusions from so small a series.

It is difficult to analyze Gruhzit's results. Microscopic technique was used, but the cells were not washed nor made up to a standard suspension; and maternal and fetal bloods were actually crossed only in the abnormal cases. The bloods of only 3 of the 86 women classed as normal were incompatible; and of these one had albuminuria and for the other two no urinalysis was made. It is difficult to understand the absence of incompatibility in view of the work of others, Halban (7), Langer (8), Schenk (9), Martin (10), Happ (11), Unger (12), McQuarrie (13), and that presented in the present paper. Thirty-one abnormal pregnancies were tabulated. In four the blood of the infants was not examined; one woman was tuberculous without toxemia. Of the 26 remaining, 14, or 53.8 per cent, were incompatible. The criteria for the differentiation of the eclamptic and nephritic types were vague in many cases; and it has not been proved that a low specific gravity of the blood rules out the eclamptic type, when checked up by post-mortem findings. In addition, such a small series, when divided up, does not lend itself to even approximate percentage analysis.

Altogether, the literature contains an investigation of 89 toxemias with a high percentage of incompatibility; and of a large number of normal pregnancies with a very low percentage of incompatibility.

In view of this, the isoagglutination relationship has been studied

in 479 mothers and their newly born infants. Although a study of the father's group would be of interest and of value, in many cases blood specimens were unobtainable and in not a few the paternity was uncertain. Consequently, this had to be omitted. If interagglutination is the prime factor in the causation of eclampsia and its prodromes, one would expect to find (1) a much higher percentage of incompatibility of the blood in such cases than in normal pregnancy; (2) an increased agglutinin titer in the maternal serum due to immunization during the prodromes of protracted cases; and (3) at least occasional evidence of specific immunization.

A number of investigators have been interested in the relationship of maternal and fetal isoagglutinins from a biological viewpoint or with regard to transfusion of the young infant; and this aspect of the present work will be reported separately, as it is not essentially germane to the problem under consideration.

METHODS

All glass-ware used was specially cleaned, and all was dry sterilized, except the hollow-ground slides and coverslips. The coverslips were used only once. The 0.85 per cent saline solution was sterilized and checked colorimetrically for approximate neutrality (pH 6.8 to 7.0). The tubes for the blood specimens contained a roughened glass rod held in the cotton plug, with which defibrination was accomplished.

The infant's blood was obtained from the maternal end of the umbilical cord immediately after delivery. The maternal blood was obtained in a dry sterile syringe by venipuncture. Both were defibrinated immediately and kept in the ice-box until used, the interval never being longer than 24 hours. Each specimen was centrifuged; the serum removed; the cells washed twice with 15 to 30 volumes of saline; packed at standard speed, about 2500 R.P.M. for 15 minutes, and then made up with saline to a 5-per-cent suspension by volume.

Microscopic technic was used routinely; a drop of serum being placed on the coverslip with a platinum loop 4 mm. in diameter and a drop of cell suspension with a 1 mm. loop. The ratio of serum to cell suspension was approximately 15–1, as determined by testing

loopfuls of dyes colorimetrically. The slip was inverted on a hollow-ground slide and sealed with liquid petrolatum. Readings were made immediately, and at the end of one hour and 24 hours. Sixteen agglutination tests were made in each case as indicated in the following schema.

	PREPARATION NUMBER															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Serum.....	M.	M.	M.	M.	F.	F.	F.	F.	2	2	2	2	3	3	3	3
Cells.....	F.	2	3	M.	M.	2	3	F.	M.	F.	3	2	M.	F.	2	3

M. = maternal; F. = fetal; 2 = known group 2; 3 = known group 3.

Readings. With the technique described one sees a varying amount of rouleaux formation, but this is readily distinguished from true agglutination by its structure, by the ease with which it breaks when the slide is tilted back and forth, and by its diminution at 37°C. The degree of agglutination was denoted by one plus, when there were a few small clumps of only a few cells each; two plus when about half the cells were in small but definite clumps, three plus when approximately three fourths of the cells were definitely clumped and four plus when there was complete agglutination. Since the ratio of serum to cell suspension was so large, and to obviate more completely any false positive readings, a one plus was not considered a positive agglutination. In the few instances in which doubt arose about the reading the preparations were repeated.

In some cases, macroscopic technique also was used to determine the agglutinin titer and the degree of hemolysis. Clean, dry-sterilized serologic tubes were used. Equal parts of fresh maternal serum or serum diluted with saline, and 5-per-cent suspension of foetal corpuscles were placed in each tube. After mixing, they were placed in the water-bath at 37°C. for 30 minutes and read; and then in the ice-box at 8°C. over night, and again read. The final dilutions of serum present in the tubes were 1–2, 1–10, 1–25, 1–50, 1–75, 1–100, 1–125, 1–150, 1–200. Control tubes containing maternal serum and maternal corpuscles, fetal serum and fetal corpuscles, and fetal corpuscles in saline were included. Hemolysis was recorded as faint trace, trace, one plus, two plus, three plus, four plus as usual. Agglu-

tination was then determined by shaking the rack until the control tubes contained a uniform suspension and then examining the other tubes in a slanting position over a light for particulate clumps. The greatest dilution producing fine definite particles indicated the titer.

RESULTS

In tabulating the results the cases have been divided into six groups; viz., (1) Normal, (2) Questionable, (3) Pre-eclamptic toxemia, (4) Eclampsia, (5) Nephritic toxemia without convulsions, and (6) Nephritic toxemia with convulsions. The differentiation is based on the history, physical examination, laboratory data, and convalescence. Nearly all the patients in Groups (1) and (2) had made frequent visits to the antenatal clinic.

These groups may be described as follows:

1. Normal—patients with no evidence whatever of toxemia.

2. Questionable—patients having any one of the following symptoms or signs: repeated nausea and vomiting after the seventh month; frequent headaches which had not been present before; repeated dizziness or visual disturbance; edema, except when the ankles only were involved; systolic blood pressure above 130 mm. on two occasions, or above 140 mm. once before the onset of labor; diastolic blood pressure above 85 mm. twice, or above 90 mm. once, before the onset of labor; a definite trace of albumin at any time before the onset of labor. This group also includes patients with cardiac decompensation, pyelitis and other infections which might emphasize the signs of toxemia. The great majority of cases were placed in this group because of elevated blood pressure, headaches or edema.

The "questionable" group does not have a place in the routine diagnosis of cases in the clinic, and would be classed as normal. We have separated them because a group of patients having even slight signs of toxemia might be expected to have an appreciably higher percentage of incompatibility, if this last is of etiological importance.

3. Pre-eclamptic toxemia—patients with *no history of preexisting symptoms or signs of nephritis or toxemia in a previous pregnancy*, but who had albuminuria and a definite elevation of blood pressure.

Edema and headache were present in the majority, and less frequently other symptoms, but these features were not regarded as essential to diagnosis. This group includes only those patients in whom *all symptoms and signs had cleared up by the time of discharge from the hospital, which was usually two to three weeks after delivery.*

4. Eclampsia—Patients who satisfy the criteria listed under pre-eclamptic toxemia, but who also have convulsions. Epilepsy, hysteria, central nervous system infections, etc., have been carefully excluded.

5. Nephritic toxemia without convulsions—patients much like those with pre-eclamptic toxemia, but *who have a history or evidence of pre-existing nephritis or toxemia in a previous pregnancy or whose symptoms have persisted after discharge from the hospital* two to three weeks after delivery.

6. Nephritic toxemia with convulsions—patients meeting the requirements of (5) above, and having convulsions in addition.

In the "normal" and "questionable" groups, the white and colored patients have been separated. Since the percentage of the different groups varies in the two races, the percentage of incompatibility might likewise vary.

Altogether, the relationship between incompatibility and toxemia has been studied in 375 women of the first two groups and 104 of the last four. The majority of the latter were patients on the obstetrical service during the period of study; several were former patients whose babies had survived and a few were patients in other hospitals or in private practice. The case records of the "former" and "outside" cases were sufficiently reliable for diagnosis on the basis outlined above. The percentage of incompatibility in each of the various groups is presented in Table I.

In Table II the data have been summarized.

Since there was no significant difference between the white and colored and the normal and questionable groups, they have all been combined as normal. The groups of pre-eclamptic toxemia and of eclampsia have been condensed, and those of nephritic toxemia and of nephritic toxemia with convulsions have been combined, since the two latter conditions may be presumed to have a common etiology.

Agglutinin titer. By means of the method outlined above, the

agglutinin titer of the serum of 33 mothers whose blood was incompatible with that of their infants was determined in 23 normal

TABLE I

GROUP	NUMBER IN EACH GROUP	NUMBER OF PATIENTS IN EACH GROUP WHOSE BLOOD WAS INCOMPATIBLE WITH THAT OF INFANT	PERCENTAGE OF PATIENTS IN EACH GROUP WHOSE BLOOD WAS INCOMPATIBLE WITH THAT OF INFANT
White. Normal	67	14	20.9
Colored. Normal	108	18	16.7
White. Questionable	100	20	20.0
Colored. Questionable	100	26	26.0
Pre-eclamptic toxemia	36	9	25.0
Pre-eclamptic toxemia (outside pts.)	4	0	0.0
Eclampsia	17	4	23.5
Eclampsia (former patients)	8	2	25.0
Eclampsia (outside patients)	12	2	16.7
Nephritic toxemia	21	2	9.5
Nephritic toxemia + convulsions	6	3	50.0

TABLE II

GROUP	NUMBER IN EACH GROUP	NUMBER OF PATIENTS IN EACH GROUP WHOSE BLOOD WAS INCOMPATIBLE WITH THAT OF INFANT	PERCENTAGE OF PATIENTS IN EACH GROUP WHOSE BLOOD WAS INCOMPATIBLE WITH THAT OF INFANT
			per cent
Normal	375	78	20.8
Pre-eclamptic toxemia	40	9	22.5
Eclampsia	37	8	21.6
Nephritic toxemia	27	5	18.7
Total normal	375	78	20.8
Total abnormal	104	22	21.1
Total normal and abnormal	479	100	20.9

women, 6 patients with pre-eclamptic toxemia, and 4 with eclampsia. In the normal women the titer varied from −1 to 10 to 1 to 125, with an average of 1 to 79; in the pre-eclamptic it varied from 1

to 25 to 1 to 125, with an average of 1 to 79; in the eclamptic it varied from 1 to 75 to 1 to 125 with an average of 1 to 109. Some hemolysis was present in the first tube of all the tests, varying from a trace to two plus, with no discernible difference between normal and abnormal. It was not considered worth while to extend the series, since an increase of titer was not found in the 10 abnormal cases. Furthermore, a marked increase in titer would be expected if pre-eclamptic toxemia and the prodromes of eclampsia were due to the repeated entrance of small amounts of fetal blood into the maternal vascular system. Such repeated injections of an antigen in an experimental animal result in much higher titers than those found.

In no case has there been any evidence of specific immunization; *i.e.*, whenever the maternal serum has agglutinated the fetal cells, the appropriate type serum invariably has done the same.

DISCUSSION

The authors quoted above, and particularly McQuarrie, have fully discussed the features and plausibility of the interagglutination theory, and little need be repeated here. The two fundamental findings on which it is based are (*a*) Flexner's description of agglutinated red blood cell thrombi in the focal liver lesions of eclampsia (13) and (*b*) Pearce's (14) production of similar lesions by the injection into dogs of immune rabbit serum strongly agglutinative of dog's corpuscles. Pearce's work has been repeated in brief, the rat being the experimental animal. Definite periportal hemorrhagic focal necroses in the liver were produced by injecting the serum of rabbits immunized to rat's corpuscles intravenously into rats. These lesions are quite similar to those found in eclampsia.

However, any theory or proof of theory of the etiology of eclampsia, besides accounting for the general symptomatology and pathology must also explain the following clinical facts:

1. Occurrence only in pregnancy
2. Approximately seventy per cent in primiparae
3. Multiple pregnancy predisposes
4. Hydramnios predisposes
5. Occurrence with hydatid mole

The interagglutination theory might well explain the occurrence of eclampsia only in pregnancy and also its greater frequency in multiple pregnancy; but it is difficult to visualize its relation to the frequency of eclampsia in primiparae and in hydramnios; and it could not possibly explain the occurrence of eclampsia with hydatid mole, since in the majority of instances of that abnormality there is no fetal blood present. It should be noted further that the fetal red blood cells are the important element, since the fetal serum is generally weak in agglutinin according to all observers, and in this series of 479 women, although 21 per cent of the maternal sera agglutinated the infants' corpuscles, never once did the fetal serum agglutinate the maternal cells.

Points of dissimilarity between eclampsia and the reaction to transfusion of incompatible blood might be adduced, but it seems unnecessary to do so here. It is especially difficult to harmonize the frequently protracted and continuous prodromes of eclampsia with interagglutination. That some sort of agglutination process may be responsible for the formation of the liver lesion in eclampsia, and perhaps for some of the symptomatology, seems quite possible and even likely, but that it is an iso-agglutination phenomenon does not appear tenable.

CONCLUSIONS

1. The isoagglutination characteristics of the bloods of 375 normal and of 104 toxemic women and their newly born infants have been studied.

2. There is no evidence that incompatibility is more frequent in toxemic than in normal gestation. Incompatibility between the bloods of mother and infant was present in 20.8 per cent of 375 normal and 21.1 per cent of 104 toxemic pregnancies.

3. There is no evidence of an increased agglutinin titer in the maternal serum of toxemic women.

4. There is no evidence of specific immunization of the mother against fetal corpuscles.

5. The discrepancy between this and previous work is probably accounted for by the size of the series studied. With a small number of cases the percentage of error and likelihood of coincidence are very great.

6. The study of this series of cases, by the methods used, gives no evidence that the late toxemias of pregnancy have their origin in isoagglutination phenomena.

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BASAL METABOLISM IN THE TOXEMIAS OF PREGNANCY[1]

H. J. STANDER, M.D., AND C. H. PECKHAM, M.D.

From the Department of Obstetrics, Johns Hopkins University and Hospital

Methods for differentiating between the various types of toxemias appearing in late pregnancy are still quite inadequate. It was with the hope of procuring a further aid for this differential diagnosis as well as of throwing more light on the nature of the toxemias that a study of the basal metabolism was undertaken.

Several investigators have recently reported on the basal metabolism in normal pregnancy in women. Magnus-Levy found that the oxygen absorption in the eighth month of pregnancy was 17 per cent higher than during the third month. Zuntz, Hasselbalch, and Carpenter and Murlin showed that at term in the normal woman the basal metabolic rate is about 4 per cent higher than in the normal non-pregnant woman in complete sexual rest. Carpenter and Murlin further showed that the heat production per unit of weight for puerperal women was 11 per cent higher than for non-pregnant women and 7 per cent higher than for the same women at term.

In 1921 Baer reported the basal metabolic rate in 44 normal pregnancies. He found that late in pregnancy the basal metabolic rate averages 33 to 35 per cent above the normal for non-pregnant women; that three days after delivery the rate is only 15 per cent above normal, and that seven to ten days *post partum* the average basal metabolic rate is approximately normal. He concludes that the increased rate in late pregnancy is due to the growing demands of the fetus and placenta, and that the incomplete or delayed return to normal is due to involution of the uterus and the onset of lactation.

In 1923 Cornell reported metabolism readings in 84 pregnant women. He found a definite increase in the metabolic rate above normal in pregnant women, his average reading before delivery being +29, and after delivery +21.5. He states that in pregnancy there is a

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gradual increase in the metabolic rate, more marked as the patient becomes older. He concludes that his results in toxic cases were so variable that no reliable information was obtained.

Sandiford and Wheeler, in 1924, studied the basal metabolism in a normal woman before, during and after a pregnancy. Their results show that there is a definite increase in total heat production during the latter part of pregnancy, beginning at the eighth month and increasing up to the time of delivery, when it is 25 per cent higher than before conception. During the puerperium there is no further increase, but a slight decrease, which the authors believe is due probably to a less active life on the part of the mother.

More recently Rowe, Alcott and Mortimer reported metabolism figures for a large series of normal pregnant women. In two series of cases they found an average basal metabolism increase during pregnancy of +0.90 and +1.02, respectively, expressed as per cent per week.

METHODS

In our studies we used the latest improved model of the McKesson Metabolor. This instrument was checked up with a Tissot Spirometer. The latter was also checked against itself from time to time, normal men being used as subjects. We feel that this type of respiration apparatus gives satisfactory results and is well fitted for a study of the problem under discussion. Tests were always conducted in the morning before the patient had had breakfast.

The basal metabolic rate was studied during pregnancy and this showed the same gradual increase during the latter half, as already reported by other authors. During this period no difference in the various types of toxemias was noted and so in the tables we record only one reading before delivery.

In Table I are given the averages for normal women. We found that the variations in the basal metabolic rate in normal women before delivery and at the various periods during the puerperium are very slight, and are therefore just recording the average figures. In Tables II, III and IV, we give the results for pre-eclamptic toxemia, nephritic toxemia and eclampsia, respectively.

As the blood pressure, albumin and oedema are generally used in

estimating the severity of the condition, we record these in the toxemia tables. It has been stated that the age of the patient has some influence on the metabolic rate, and for this reason we give this information. The parity and, in the case of eclampsia, the number of convulsions before delivery and after delivery, respectively, are also noted.

In Figure 1 we have charted the average values. These curves indicate at a glance the changes that take place before delivery and in the puerperium, as well as to what extent the toxemias are associated with any variations in the basal metabolic rate.

TABLE I

Normal pregnancy

NUMBER OF CASES, 13	BASAL METABOLIC RATE							
		Puerperium						
	Before delivery	1st day	3rd day	5th day	7th day	10th day	15th day	Discharge
Limits.....	−8 to +15		+2 to +16		+4.5 to +7	−1.5 to −1		
Averages....	+4.5	+8.5	+10.8	+9	+5.75	−1.25		

DISCUSSION

Normal pregnancy. Our average value for normal pregnant women just before delivery is +4.5, a value which is slightly lower than those reported by others. There certainly is a definite tendency for the basal metabolic rate in pregnancy to be at the upper margin of, or slightly above, the normal non-pregnant limits. The puerperium shows a steady, gradual return to normal within 10 days. This agrees fairly closely with the findings of Baer, and he is probably correct in assuming that the increased rate in late pregnancy is due to the growing demands of the fetus and placenta. It should be borne in mind, however, that a slight increased activity of the thyroid might play a rôle here.

Pre-eclamptic toxemia. This group starts with a basal metabolic rate, of +23, just before delivery. The rate begins to decrease on the

TABLE II

Pre-eclamptic toxemia

CASE NUMBER	AGE	COLOR	PARITY	WEIGHT		BLOOD PRESSURE	ALBUMIN	EDEMA	BASAL METABOLIC RATE							
				Admission	Discharge				Before delivery	Puerperium						
										1st day	3rd day	5th day	7th day	10th day	15th day	Discharge
22	19	W.	I	153½	112½	160/114 – 108/76	1+–trace	Slight	+37	+16.5	+16	+12.5	+6.5	+3	−1	−4
12	17	B.	I	154¼	91	184/100 – 112/45	12–trace	Marked	+1.5	+10.5	+13.5	+10		−6	−14.5	−9
11	20	W.	I	Not obtained	148	196/92 – 120/65	2.5–0	Slight			+35	+40	+42	+23		+23
10	25	W.	I	Not obtained	131	112/60 – ?	1.5–0	Slight			+35	+14	+5	−0.5		−0.5
4	26	B.	III	148½	134½	218/140 – 130/75	0.75–trace	None	+23	+41	+29.5		+8	−3	+8	+8
44	46	B.	VIII	172	?	222/102 – 132/96	Trace–0	Moderate	+36							
39	20	W.	II	152	122½	200/134 – 120/84	2.6–trace	Slight	+15	+8.5		+12	+3.5			+3.5
1	34	B.	VI	193	168¾	220/135 – 138/90	0.5–0	Moderate	+23					+12	+10	+10
38	23	W.	V	170	147	202/128 – 135/86	9.0–trace	Slight	+3	+12		+7	+6	+6	−5.5	−7
42	35	B.	XII	133¼	117½	180/103 – 154/98	Trace–0	Slight	+18				+7	+5	+12	+12
43	22	W.	I	146¼	131	154/104 – 126/78	2.0–trace	Moderate	+21							

40	18	B.	I	148½	124½	$\frac{210}{100} - \frac{130}{84}$	0.1–0	Slight	+28	+14						
37	23	B.	IV	147½	117½	$\frac{200}{120} - \frac{102}{68}$	3.0–0	Moderate	+35				+0.5			
30	22	B.	II	140	?	$\frac{154}{108} - ?$	0.5–0	Slight	+39.5							
29	31	B.	II	173½	?	$\frac{142}{90} - \frac{120}{80}$	Trace–0	Slight	+36							
16	30	B.	I	162	138	$\frac{144}{100} - ?$	0 –0	Slight	+18				+16	+22		
36	31	B.	I	130	112	$\frac{180}{100} - \frac{130}{86}$	0.2–0	Slight	+5	+10		+11				
Average									+23	+16.1	+25.8	+15.2	+13.3	+6.8	+1.5	+4

TABLE III

Nephritic toxemia

CASE NUMBER	AGE	COLOR	PARITY	WEIGHT		BLOOD PRESSURE	ALBUMIN	EDEMA	CONVULSIONS		BASAL METABOLIC RATE							
												Puerperium						
				Admission	Discharge				Before delivery	After delivery	Before delivery	1st day	3rd day	5th day	7th day	10th day	15th day	Discharge
41	23	B.	II	204	181½	$\frac{210}{140} - \frac{164}{110}$	4–trace	Moderate	0	0	+26.5		+27	+29	+13	+10.5	+16	+16
34	33	W.	X	191½	?	$\frac{200}{140} - \frac{175}{135}$	2–0.5	Slight	0	0	+39							
27	32	W.	IV	132½	?	$\frac{160}{124} - \frac{135}{95}$	1–0	0	0	0	+16							
21	23	W.	I	183½	157	$\frac{205}{124} - \frac{134}{84}$	8–trace	Moderate	2	0	+42.5	+22	+16	+15	+2	+1	−4.5	−1.5
9	37	W.	VIII	150½	132½	$\frac{184}{118} - ?$	?–trace	Slight	0	0	+56	+47	+35.5	+35.5	+40	+32.5		+32.5
5	31	W.	VI	161½	149½	$\frac{200}{130} - \frac{145}{105}$	1–0	0	0	0	+18						+3	+3
Average											+33.0	+34.5	+25.3	+26.5	+18.3	+14.7	+4.8	+12.5

TABLE IV

Eclampsia

CASE NUMBER	AGE	COLOR	PARITY	WEIGHT		BLOOD PRESSURE	ALBUMIN	EDEMA	CONVULSIONS		BASAL METABOLIC RATE							
												Puerperium						
				Admission	Discharge				Before delivery	After delivery	Before delivery	1st day	3rd day	5th day	7th day	10th day	15th day	Discharge
35	30	B.	IV	205¾	153	$\frac{190}{120}-\frac{108}{75}$	10–0	Marked	0	10	+24		+31.5	+70	+24.5	+19	+14	−6
32	25	W.	I	144¾	124½	$\frac{170}{106}-\frac{96}{68}$	15–0	Slight	2	0		+30	+25	+35	+8	−6.5	−3	−3
31	36	W.	IV	140	105	$\frac{220}{120}-\frac{116}{82}$	28–trace	Slight	14	0	+41				+38	+10.5	+0.5	−5
23	29	W.	I	151	102½	$\frac{200}{104}-\frac{116}{74}$	6–trace	Medium	3	4			+33	+42.5	+18	+14.5	+10	+1
Average											+32.5	+30	+29.8	+49.2	+22.1	+12.5	+5.4	−3.2

third day of the puerperium and gradually drops, until it reaches the normal level on the fifteenth. It is the same type of curve as that for normal pregnancy, being just slightly more elevated. From these curves it would not be possible to make a definite differentiation between normal pregnancy and pre-eclamptic toxemia.

Nephritic toxemia. The nephritic type averages +33 before delivery, a basal metabolic rate which is decidedly above the normal non-

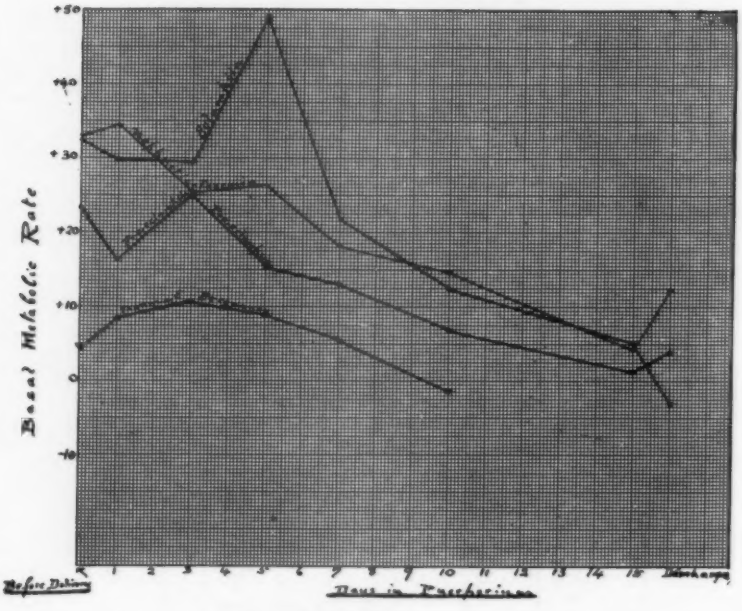

FIG. 1

pregnant figure. The return to normal starts almost immediately after delivery and reaches the zero line by the fifteenth day of the puerperium. The curve is slightly higher than that for the pre-eclamptic group.

Eclampsia. We have had considerable difficulty in obtaining the basal rate of certain eclamptic patients. When the patient is in coma, or violent, it is almost impossible to obtain satisfactory results. The

curve in Fig. 1, constructed from the few readings we have been able to obtain, agrees very closely with the nephritic toxemia curve. There is a decided peak on the fifth day of the puerperium. The return to normal is again steady and gradual. We have not noticed any parallelism between the basal metabolic rate and the blood pressure, or the amount of albumin and oedema, or the number of convulsions. It is true that as the convulsions cease, and as the blood pressure decreases and the albumin and oedema disappear, the patient gets well and her basal rate also returns to normal; yet the basal metabolic rate does not necessarily indicate the severity of the condition.

CONCLUSIONS

1. In the latter half of pregnancy we have noticed a definite elevation in the basal metabolic rate and a return to normal at about the tenth day of the puerperium. The return is gradual and steady.

2. In pre-eclamptic toxemia the basal metabolic rate is slightly higher than in normal pregnancy. It returns to a normal level at about the fifteenth day of the puerperium.

3. Nephritic toxemia is associated with a basal metabolic rate of about +33 before delivery. This figure is slightly higher than that for the pre-eclamptic group. The tendency for the basal rate in nephritic toxemia is to stay elevated for a longer time during the puerperium than it does in pre-eclampsia.

4. The basal metabolic curve for the eclamptic group is almost identical with the nephritic curve.

5. Basal metabolism determinations do not prove of any real help in differentiating between the various types of toxemias in late pregnancy.

6. In normal pregnancy, as well as in the different toxemias of pregnancy, there is some factor or factors, which result in an elevated basal metabolic rate as term is approached, a gradual return to normal ensuing during the puerperium. It is probable that such factor, or factors, may have to do with the growing product of conception, as well as a slight increased activity of the thyroid gland.

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THE FETAL SYNTHESIS OF FATS[1]

LAURENCE G. WESSON

From the Laboratory of Physiological Chemistry, the Johns Hopkins University

The question of the placental transmission of fats from the maternal to the fetal circulation is an interesting one, for upon its solution depends the answer to the question as to whether or not there is already developed in the fetus the mechanism for the transformation of the carbohydrates into fats.

Ahlfeld (1), Oshima (2), Murlin and Bailey (3), and Slemons and Stander (4), who attacked this problem from the point of view of the fat contents of the maternal and fetal bloods, found a large and inconstant difference between these values. This led them to the conclusion that the fats of the fetus are not derived from the maternal circulation. Their results probably more surely indicate that the fats at least are not transmitted by a diffusion process similar to that of the placental transmission of glucose. However, a diffusion process of this sort is scarcely to be expected, but rather a lipolytic sort of transmission resembling that of the intestinal epithelium.

Gage and Gage (5) and Mendel and Daniels (6) arrived at the same conclusion as did the above mentioned authors after a series of experiments in which Sudan III was fed along with fats to pregnant animals. No trace of the dye could be found in the fetuses from these animals, as would be expected, they maintain, if the fetal fat had been derived from the mother. According to Cunningham (7) and others, the placental barrier exhibits a remarkable selective resistance to certain types of substances to a degree which exceeds that observed in other physiological membranes. Transmission of the fat would therefore be conceivable without the accompanying transmission of its dissolved dye, whereas in the usual case transmission of the dye has been considered as being equivalent to transmission of the fat.

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Thiemich (8) fed a bitch during one pregnancy a fat of low iodine number, and during a succeeding one a fat of high iodine number, but found the iodine value of the fats of the new-born pups to be the same in each case. His conclusion from this experiment was that the fetus did not derive its fat from the mother. Later (9) he modified this conclusion on the basis of an analysis of the adipose tissue of another bitch and total fats of the pups. The values for the iodine number of these fats led him to believe that, during the intrauterine development, the fetus derived part of its fat at least from the mother.

Hofbauer (10) also concluded from histological and chemical evidence that fats are transmitted from mother to fetus. The examination of placental villi from various sources showed layers of fat droplets which Hofbauer compares to similarly appearing layers of known transmitted fat in the intestinal mucosa. The presence of lipases in these tissues also makes the resemblance to intestinal transmission the more striking. Since fetally-formed fat might be expected to be distributed in the tissues nourished by the fetal circulation, and since lipases or esterases are found in most, if not all, tissues, evidence of this nature for or against placental transmission of fats would appear to have little weight. The chemical evidence adduced by Hofbauer is of diverse nature. Feeding experiments with several fats stained by Sudan and alkanna red, gave, according to Hofbauer, traces of the dyes in the blood of the new-born animals. Contrary results were obtained with stained stearic acid which gave no dye in the fetal blood. Sesame oil and cocoa butter were likewise fed and tested for in the fetal fats,—the first gave negative, but the second (cocoa butter) positive results. The method which was used for identifying cocoa butter by means of the lauric acid which it contains is open to criticism, however, since the separation of lauric acid from other volatile fatty acids by means of its barium salt is not sufficiently characteristic, and the fatty acid separated in this way from the other fatty acids present in the fetal fat was not otherwise identified. Moreover, no experimental results of the examination of the fetal fat for lauric acid are given. These results, therefore, appear to be of an inconclusive nature.

In the work described in the present paper, the fat was identified,

instead of by its iodine number, by the amount of precipitate given by the highly unsaturated acids of the fat with bromine, according to the method of analysis used in a previous work (11) for the comparative estimation of the highly unsaturated acid, arachidonic acid, in animal tissues. The fats used were cod-liver oil of good quality, which contains a large percentage of highly unsaturated acids, and butter fat, which contains a low percentage of highly unsaturated acids. One group of rats was fed cod-liver oil as a percentage of its rations before and during pregnancy, while the other group was fed butter fat for the same period. The fetuses of both groups were removed from the mother just before birth, and were analyzed for the highly unsaturated acids in their fats. No appreciable difference was found between the groups as regards the content of highly unsaturated acids of the fetuses, although a marked contrast was noted between the groups as regards the corresponding values for the mothers.

The first group of rats before mating was maintained for several months on the following diet: corn starch, 48; rolled oats, 40; technical casein, 5; calcium carbonate, 1.5; sodium chloride, 1; cold-pressed Newfoundland cod-liver oil (Mead and Johnson's), 5; iodine in small amounts in the drinking water. The other group was fed a diet in which butter fat was substituted for the cod-liver oil. On the 20th or 21st day after mating (approximately the end of the period of gestation), the fetuses were removed and analyzed by the method above referred to.

In the following tables, the content of highly unsaturated acids per gram of moist tissue is calculated as arachidonic acid from the weight of the bromine addition products of these acids. The factor used is the theoretical one, 0.3225. The "ratio" is the ratio of the calculated highly unsaturated acids to the ether extract content of the moist tissues.[2]

It is seen by an examination of Tables I to IV that the values for the unsaturated acids in the fetuses are approximately equal for both groups. If the fats had been derived in large part from the

[2] The "ratio" of highly unsaturated acids to ether-soluble substances in the cod-liver oil used was 0.210, while that of the butter fat was 0.00309, or 1/70th as large.

maternal circulation, we might well expect the fetuses from the mothers which were fed unsaturated fats (Table I) to have two or three times

TABLE I

Analysis of the whole tissues of fetuses from mothers which were fed cod-liver oil with the diet before and during gestation

FROM RAT		WEIGHT	UNSATURATED ACIDS	ETHER EXTRACT	RATIO
no.		*grams*	*pro mille*	*pro mille*	
1	Eight fetuses	38	0.61	20	0.030
2	Six fetuses	18	0.43	26	0.016
3	Six fetuses	23	0.40	39	0.010
4	Nine fetuses	23	0.52	20	0.026
Average.....		26	0.49	26	0.021

TABLE II

Analysis of the whole tissues of fetuses from mothers which were fed butter fat with the diet before and during gestation

FROM RAT		WEIGHT	UNSATURATED ACIDS	ETHER EXTRACT	RATIO
no.		*grams*	*pro mille*	*pro mille*	
5	Twelve fetuses	17	0.26	12	0.021
6	Ten fetuses	45	0.35	19	0.019
7	Ten fetuses	52	0.42	12	0.034
8	Nine fetuses	14	0.45	16	0.028
9	Eight fetuses	16	0.51	21	0.025
Average.....		29	0.40	16	0.025

TABLE III

Analysis of the whole tissues of the mothers of fetuses of Table I. These rats were fed cod-liver oil in the diet

RAT	WEIGHT	UNSATURATED ACIDS	ETHER EXTRACT	RATIO
no.*	*grams*	*pro mille*	*pro mille*	
2	106	2.17	48	0.041
4	110	2.58	62	0.042
Average.....	108	2.38	55	0.042

* The analyses of rats, numbers 1 and 3, were unfortunately lost in the course of the procedure.

or more as high a content of unsaturated acids as the fetuses whose mothers were fed a saturated fat (Table II). If transmission of

fats or fatty acids has occurred across the placental membrane, we must postulate a type of transmission which is highly selective as regards the degree of unsaturation of the transmitted fats or fatty acids; or we must assume a mechanism for hydrogenating and dehydrogenating in the fetus which would form, from either type of fat or fatty acid, a similar amount of highly unsaturated acids per gram of fetal tissue or per gram of total ether-soluble substances. Both of these assumptions appear to be less plausible than the assumption that the fetus has been able to synthesize the fatty acids in amount which satisfies its own requirements.

TABLE IV

Analysis of the whole tissues of the mothers of fetuses of Table II. These rats were fed butter fat in the diet

RAT	WEIGHT	UNSATURATED ACIDS	ETHER EXTRACT	RATIO
no.	*grams*	*pro mille*	*pro mille*	
5	188	0.93	83	0.011
6	(174)	(0.31)	(58)	(0.0052)
7	161	0.93	67	0.014
8	93	1.02	90	0.011
9	176	0.73	76	0.0096
Average.....	155	0.90	79	0.011

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A NOTE ON THE PROPHYLACTIC AND THERAPEUTIC ACTIVITY OF THE SODIUM SALT OF ACETYL OXYAMINOPHENYLARSINE ACID (STOVARSOL) IN EXPERIMENTAL SYPHILIS OF RABBITS[1]

ALLAN K. POOLE, M.D.

From the Department of Medicine of the Johns Hopkins Medical School

INTRODUCTION

This report deals with the prophylactic and therapeutic activity of the sodium salt of acetyl oxyaminophenylarsine acid in experimental syphilis of the rabbit. The compound is a pentevalent arsenical, and within the last few years has been extensively advertised in European journals, under the trade name "Stovarsol," as a prophylactic and therapeutic agent for use in syphilis. It is related to atoxyl. A noteworthy feature of the preparation is that its treponemicidal action is manifested after oral administration.

The experimental studies of the drug have not, thus far, been numerous, and further confirmation of its therapeutic and toxic properties when given by mouth would seem to be highly desirable before the drug is extensively employed in clinical work. It was for this reason that the present study was undertaken.

HISTORICAL

The compound was first synthesized by Ehrlich (1), being known as No. 594, but was discarded by him as being unsatisfactory. It was again synthesized in 1921 by Treufouel, working under the direction of Fourneau (2). Its treponemicidal activity in experimental syphilis in the rabbit and monkey, and its therapeutic activity in human syphilis, were the object of study by Levaditi, Navarro-Martin, Guenot and Schwartz (3). These investigators concluded that the drug, after administration by mouth, exerts a prophylactic and therapeutic action in rabbits and monkeys, experimentally infected with

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syphilis. Likewise in syphilitic human beings, the oral administration of the compound brought about healing of the cutaneous lesions together with disappearance of treponemes from the lesions, and, in two human volunteers experimentally infected, appeared to exert a prophylactic effect. In rabbits, the drug was found to be toxic in doses of 0.66 gram per kilogram, administered by mouth, but was said to be well tolerated in doses of 0.3 to 0.4 gram per kilogram.

Following the appearance of this work, the drug was subjected to clinical trials in Germany with conflicting results. For a recent summary of this phase of the question the reader is referred to a paper by Luerssen (4) in which a complete bibliography is given. Since the present investigation was undertaken, two experimental studies of the drug have appeared, one by Kolle (5), the other by Worms (6).

Kolle found that the drug is toxic for rabbits when given by mouth in the doses recommended by Levaditi (0.2 to 0.3 gram per kilogram), and that it is more toxic when administered by mouth than when given intravenously or subcutaneously. He pointed out that the animals which had received the drug as a prophylactic, and in which no syphilitic lesions subsequently developed, should be subjected to reinoculation and their organs studied for the presence of treponemata before concluding that infection had really been prevented.

It should be noted that at the time our study was begun Kolle's article had not come to our attention, and, as will be seen from the plan of the investigation, the same opinion was held by us as regards the necessity of more complete study of the experimental animal before arriving at the conclusion that the drug exerts a sure prophylactic effect. In fact, this investigation was undertaken with the express purpose of subjecting the drug to as severe a test, both from the prophylactic and therapeutic viewpoints, as our present knowledge of experimental syphilis permits.

Worms was unable to obtain uniform curative or prophylactic effects in rabbits infected with Treponema cuniculi or three different strains of Treponema pallidum, when Stovarsol or its German equivalent, Spirocid, was administered by mouth in doses ranging for the most part between 0.17 and 0.33 gram per kilogram. In fact, in his hands there were more failures than cures. He likewise found great variation in the toxicity of the drug and is of the opinion that it is

not suited for use in rabbits. As will be seen later, our experience with the drug in so far as toxicity is concerned is in complete agreement with that of Worms.

Within the last few months several articles on the treatment of malaria and amoebic dysentery with this preparation have appeared in the French and American literature (7, 8, 9).

Through the courtesy of Dr. Fourneau of Paris we were furnished with a supply of the drug that we might carry on experimental studies, and we desire at this point to acknowledge our indebtedness to him.

EXPERIMENTAL

The experiments to be reported were designed to elicit information on the following points: (1) prophylactic value; (2) therapeutic value; (3) toxicity of the drug. Only adult male rabbits were used. The Nichols strain of Treponema pallidum was employed throughout. The drug was administered in 10 per cent aqueous solution by means of a stomach tube, the animal being lightly etherized for this procedure. All the animals were starved for 24 hours prior to and following treatment.

Experiment I. Prophylactic value

Two groups, of six and four rabbits, respectively, were used. Each animal was inoculated intratesticularly with 0.2 cc. of an emulsion of syphilitic rabbit testis containing numerous actively motile treponemata.

Group A. Test group. Six rabbits were given 0.5 gram per kilogram of the drug by mouth, as described above, two hours after inoculation. This dose, while admittedly close to the toxic dose as described by Levaditi, was selected because it was thought that, if the drug failed to show a prophylactic effect when subjected to a rigid test with such large doses, it would be needless to study the action of smaller doses.

Group B. Control group. Four rabbits were kept under the same conditions as Group A, but received no Stovarsol.

The animals in each group were observed for a period of 120 days. Wassermann reactions were performed on the blood serum of these

animals at frequent intervals.[2] Sixty-six days after inoculation such animals in Group A as had survived were etherized and the inoculated testis and both popliteal lymph nodes were excised, emulsified in physiological salt solution and injected into two normal male rabbits. The animals to which transfer was made received 0.4 cc. of testicular emulsion in one testicle and 0.4 cc. of gland emulsion in the opposite testicle. Testis and node transfer was carried out likewise in the two animals which died on the 51st and 66th day, respectively, but not in the case of the two animals dying on the 21st day, since post-mortem change was too far advanced.

In addition to the tissue transfers, the surviving animals in Group A were reinoculated in the remaining uninoculated testis with 0.3 cc. of emulsion of syphilitic rabbit testis containing actively motile treponemata. These reinoculations were carried out 78 days after the original inoculation. Tissue transfers were not performed in the case of the control group as they were all showing active syphilitic lesions at the time.

The results of the experiment are shown in Table I.

Results. Table I shows that in the untreated animals syphilitic orchitis developed in each instance in the inoculated testis; furthermore, clinical evidence of systemic disease was manifest in each animal, indicating that the inoculum was highly virulent. In addition, the blood Wassermann became positive and remained so for at least as late as 92 days after inoculation.

In the test group no clinical evidence of orchitis was manifest in any of the animals during the period of observation, nor did any of the animals develop a positive blood Wassermann reaction. Two of the animals (Nos. 2 and 4) died on the twenty-first day after inoculation, and necropsy suggested that these deaths were due to drug intoxication. There was no macroscopic evidence of syphilis in the inoculated testes, and dark field examination of these organs revealed no treponemata.

[2] The technic employed in performing the Wassermann reaction in these experiments will not be presented here. In a forthcoming paper it will be given in full. It will suffice to say that as performed in this laboratory the reaction is almost always negative in normal rabbits but positive with remarkable constancy in early active untreated syphilis of the rabbit.

TABLE I

Prophylaxis experiment

RABBIT NUMBER	OCCURRENCE OF ORCHITIS	OCCURRENCE OF GENERALIZED LESIONS	WASSERMANN REACTION: Day of disease 17	24	30	39	48	64	76	Reinoculation on 78th day	92	120	RESULT OF LYMPH NODE TRANSFER	RESULT OF TESTIS TRANSFER	REINOCULATION ON 78TH DAY. RESULT	REMARKS
Group A. Prophylactic group. Treatment 2 hours after inoculation																
1	None	None	0	0	0	0	0	0		Died			Negative	Negative	Not done	Died under ether 66th day
2	None	None	Ac.	Died									Not done	Not done	Not done	Died 21st day
3	None	None	0	0	0	0	0	0	0	Reinoculation	0	4	Negative	Negative	Positive	Observed 120 days
4	None	None	0	Died									Not done	Not done	Not done	Died 51st day
5	None	None	0	0	0	0	0	0	0	Reinoculation	0	4	Negative	Negative	Positive	Observed 120 days
6	None	None	0	0	0	0	0	Died					Negative	Negative	Not done	Died 51st day
Group B. Control group																
7	Positive 17th day	Metastatic orchitis; bone lesions	0	4	4	4	4	4	4		4		Not done	Not done	Not done	Discarded after 98 days
8	Positive 24th day	Metastatic orchitis; bone lesions	0	0	4	4	4	4	4		4		Not done	Not done	Not done	Discarded after 98 days
9	Positive 17th day	Keratitis; bone lesions	0	0	4	0*	4	4	4		4		Not done	Not done	Not done	Discarded after 98 days
10	Positive 17th day	Metastatic orchitis	0	0	4	4	4	4	4		4		Not done	Not done	Not done	Discarded after 98 days

Note: Wassermann reaction results: 0 = negative; 1 = 25 per cent fixation; 2 = 50 per cent fixation; 3 = 75 per cent fixation; 4 = 100 per cent fixation; Ac. = anticomplementary serum.

* At this point orchitis was subsiding and Wassermann reaction became negative. Subsequently there was recrudescence of the orchitis with recurrent positive Wassermann reaction.

One animal (No. 6) died on the fifty-first day after inoculation. Here, also, there was no macroscopic evidence of syphilis and no treponemata were found. One animal (No. 1) died under ether anesthesia on the sixty-sixth day after inoculation while the testis and lymph nodes were being removed. No treponemata could be found in the inoculated testis.

The two animals which survived (Nos. 3 and 5), and were reinoculated on the seventy-eighth day after the original inoculation, developed clinical signs of orchitis 20 days after reinoculation. Characteristic examples of Treponema pallidum were demonstrated in the inoculated testes and the blood Wassermann reaction became positive.

In none of the rabbits inoculated with the lymph nodes and testes from four of the six animals in Group A (Nos. 1, 3, 5, and 6) was there any indication of syphilitic infection.

Experiment II. Curative Action

Two groups of six and four animals, respectively, were utilized. All the animals were inoculated intratesticularly on the same day with 0.2 cc. of emulsion of syphilitic rabbit testis, containing actively motile treponemes. Primary syphilitic orchitis developed in each instance in from 17 to 24 days after inoculation.

Group A. Twenty-eight days after inoculation, when the orchitis was manifest, all the animals in this group were given a single dose of 0.5 gram per kilogram of the drug by mouth. Wassermann reactions were performed at frequent intervals on the blood serum of these animals. As will be seen later, all the treated animals died within 27 days after treatment. In three of them the inoculated testis and both popliteal lymph nodes were emulsified in physiological salt solution and transferred to normal animals. This was not done in the case of the other three because of advanced post-mortem changes.

Group B. Control group. Four rabbits were utilized, and kept under the same conditions as Group A, but received no Stovarsol.

The results are shown in Table II.

Results. Table II shows that in the test group the syphilitic orchitis was completely healed between the 11th and 20th day after the administration of a single dose of the drug. No attempt was

TABLE II

Therapeutic experiment

RABBIT NUMBER	INCUBATION PERIOD	DURATION OF ORCHITIS AFTER TREATMENT	WASSERMANN REACTION Day of disease							RESULT OF LYMPH NODE TRANSFER	RESULT OF TESTIS TRANSFER	REMARKS
			15	26		32	39	46	53			
Group A. Test group. Treatment 28 days after inoculation												
	days	*days*										
1	17	20	0	4	Treatment on 28th day	4	2	2		Not done	Not done	Died 20th day after treatment
2	17	16	0	4		0	0	0	0	Negative	Negative	Died 27th day after treatment
3	17	20	0	0		4	2	2		Negative	Negative	Died 23rd day after treatment
4	20	11	0	4		4	4	4		Not done	Not done	Died 20th day after treatment
5	20	11	0	4		4	4	4	4	Negative	Negative	Died 27th day after treatment
6	17	16	0	4		Ac.	Ac.			Not done	Not done	Died 16th day after treatment
Group B. Control group												
7	17	No treatment	0	4		4	4	4	4	Not done	Not done	Generalized syphilis. Discarded after 98 days
8	24	No treatment	0	0		4	4	4	4	Not done	Not done	Generalized syphilis. Discarded after 98 days
9	17	No treatment	0	0		4	4	4	4	Not done	Not done	Generalized syphilis. Discarded after 98 days
10	17	No treatment	0	0		4	4	4	4	Not done	Not done	Generalized syphilis. Discarded after 98 days

Note: Wassermann reaction results: 0 = negative; 1 = 25 per cent fixation; 2 = 50 per cent fixation; 3 = 75 per cent fixation; 4 = 100 per cent fixation; Ac. = serum anticomplementary.

made to follow the disappearance of treponemata from the lesions for fear of altering the course of the disease. In two of the animals the Wassermann reaction was unchanged, remaining positive up to the time of death—20 and 27 days, respectively, after treatment. In two others the reaction became doubtful. In the remaining two, one became anticomplementary and one became completely negative after treatment. All six of these rabbits died between the 16th and 27th day after treatment. Necropsy was done on all animals, but no macroscopic evidence of syphilis could be found, either in the inoculated testis or the internal organs, nor could treponemata be demonstrated in the inoculated testis.

In the three animals in which the inoculated testis and popliteal lymph glands were removed immediately and transferred to normal animals, no treponemata could be demonstrated in the inoculated testis, and none of the animals inoculated with testis and lymph glands from these treated rabbits developed syphilis within 90 days after inoculation.

In the control group, however, all the animals survived and the disease manifested itself in the usual manner, each animal showing systemic lesions and exhibiting a positive Wassermann reaction throughout the period of observation.

The high mortality in the animals treated with Stovarsol was almost certainly due to the drug, and suggested that the dose employed was above the maximum tolerated dose. It seemed advisable, therefore, to determine, if possible, the maximum tolerated dose in rabbits. Accordingly, a toxicity experiment was carried out as follows.

Experiment III. Toxicity experiment

Fourteen normal adult male rabbits were given the drug by mouth precisely as described previously, in amounts ranging from 1 to 0.2 gram per kilogram. The doses employed were 1, 0.75, 0.6, 0.5, 0.4, 0.3, and 0.2 gram per kilogram, and each amount was given to two rabbits. The animals were observed for 90 days. Necropsies were performed on all that died.

The results are shown in Table III.

Results. Of the fourteen animals in this group receiving Stov-

arsol, twelve died within 20 days after treatment. The two surviving animals received 0.6 and 0.3 gram per kilogram, respectively. In general those receiving larger doses died earlier than those receiving smaller doses. It is evident that the experiment did not disclose the minimum lethal dose of the particular sample of the drug employed, nor did it give any information as to the maximum tolerated dose. Levaditi and his co-workers (3) stated that the drug was well tolerated in doses of 0.3 to 0.4 gram per kilogram. Our experiments would indicate that for some samples of the drug the toxic level may fall below that ascribed to the drug by other workers and our findings in this respect agree with those of Kolle.

TABLE III

Toxicity experiment

DOSAGE IN GRAMS PER KILOGRAM	NUMBER OF RABBITS TREATED	NUMBER OF DEATHS
1.0	2	2 (9, 15)
0.75	2	2 (13, 14)
0.6	2	1 (18)
0.5	2	2 (10, 14)
0.4	2	2 (19, 24)
0.3	2	1 (18)
0.2	2	2 (18)

Note: Figures in parenthesis indicate number of days after treatment on which death occurred.

There is little doubt that death in these animals was due to Stovarsol poisoning. The first symptom noted in these animals was anorexia, followed by lethargy, and a few days before death profuse watery diarrhea was noted. There was no evidence of blindness. Autopsy was performed on all the animals that died, both in this and in the preceding experiments. In all cases the stomach was found to be enormously distended with food, sometimes more than half filling the abdominal cavity. One animal showed marked jaundice, and several exhibited what appeared to be a terminal pulmonary congestion. The intestines were injected in most cases but no hemorrhages were seen. No macroscopic changes in the liver or kidneys were noted. Histologic study of the tissues has not as yet been carried out.

No control animals were used in this group as the large number of rabbits in the laboratory at this time served as controls. There was no epidemic disease present and the mortality among the other laboratory rabbits was exceedingly low.

DISCUSSION

In regard to the prophylactic value of the preparation the results of the experiment cited above are fairly clear cut. Under the conditions outlined, the sodium salt of acetyl oxyaminophenylarsine acid, administered to rabbits by mouth in single doses of 0.5 gram per kilogram within two hours after intratesticular inoculation with a virulent strain of Treponema pallidum, appears to be able to prevent the development of syphilitic lesions at the site of inoculation. Furthermore, such animals show no clinical signs of systemic infection, they do not develop positive Wassermann reactions in the blood, and they do not harbor the infection in distant lymph nodes. In addition, they are susceptible to a second inoculation with the same virus. These facts warrant the statement that according to the most rigid biologic tests that can be devised at present the drug exhibits a definite prophylactic action when administered by mouth to rabbits in doses of 0.5 gram per kilogram immediately after inoculation with active virus.

The results of the attempt to determine the therapeutic activity of the preparation in rabbits with well-advanced syphilitic infection were not so decisive, owing to the premature death of all the treated animals. However, under the conditions of the experiment, a single dose of the drug was able to bring about rapid regression of a syphilitic orchitis which was in the act of developing rapidly when the drug was administered. In addition, the Wassermann reaction was altered toward the negative side in most of the animals, although the period of observation was too short to permit of any deductions as to the final outcome of the reaction. The same may be said of the infection itself. Whether a complete sterilization of the treated animals was effected or not will have to remain undecided by reason of the premature death of the animals. However, it is, we believe, significant that, in the three treated animals in which lymph node and testis transfer to normal animals could be carried out, there was no evidence

of any syphilitic infection. According to rigid criteria of cure (10), these animals had been cured, although it is freely admitted that the period of observation was far too short. On the whole the experiment suggests that the curative action of the drug, in the amount used, parallels its prophylactic action.

It is clear from the experiments that the dose employed, namely, 0.5 gram per kilogram, was well above the lower limit of toxicity. Our attempt to determine the maximum tolerated dose leads us to believe that at least some samples of the drug are more toxic than Levaditi's observations would indicate. Indeed, in the single toxicity experiment which we conducted, we were not successful in disclosing the maximum tolerated dose. Even 0.2 gram per kilogram given by mouth killed both the animals receiving this amount, the lowest dose tested, and an amount which, according to Levaditi, is well borne by rabbits.

It is clear that much further investigation of this compound is necessary to determine its chemotherapeutic index in experimental syphilis, and it seems highly desirable to determine this factor with a reasonable degree of certainty before advocating the indiscriminate use of the preparation as a prophylactic agent to be used by the laity after exposure to syphilitic infection. This is not the place to discuss in general the propriety of using antisyphilitic remedies administered by mouth, intravenously or subcutaneously, to prevent infection in exposed persons. The danger of suppressing the initial phases of the disease without eradicating the infection, thus leading to a false sense of security on the part of both the patient and the physician, has already been abundantly stressed elsewhere. It should be sufficient at this point to call attention to the need of more extensive studies of this particular drug from the experimental standpoint. The compound does possess marked treponemicidal properties when given by mouth to experimentally infected rabbits in doses which, however, are likely to be lethal. Whether this same activity is regularly manifested after the exhibition of much smaller doses, which can be shown to be safe, remains to be seen. In any event the development of the treponemicidal activity following oral administration is an interesting fact, and perhaps worthy of further investigation.

CONCLUSIONS

1. The sodium salt of acetyl oxyaminophenylarsine acid given by mouth in a single dose of 0.5 gram per kilogram appears to exert a definite prophylactic action in experimental syphilis of the rabbit.

2. In the same dosage, it apparently can bring about the healing of the initial syphilitic orchitis.

3. The drug is toxic for rabbits in single doses as low as 0.2 gram per kilogram, when administered by mouth, causing the death of the animal within 27 days or less.

4. Further experimental study of the drug is advisable before it is employed in human syphilis.

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